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ENVIRONMENTAL FATE STUDIES ON CERTAIN MUNITION WASTEWATER CONSTITUENTS

Final Report, Phase II — Laboratory Studies

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The major transport and transformation processes affecting the environmental fate of TNT, 2,4-DNT, RDX, and trinitroglycerin were identified. Rate and equilibrium constants for these processes were determined from laboratory studies. These constants were incorporated into a computer model that was programed to simulate the Holston River, the New River, and Waconda Bay. From these simulations, concentration-time dependent curves were constructed to estimate munition component concentrations at various locations in the receiving water body.

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CONTENTS

TABLES

ILLUSTRATIONS

EXECUTIVE SUMMARY

Laboratory studies were conducted to estimate the rate constants and half-lives for the photochemical and microbial transformations of TNT, RDX, 2,4-DNT, and trinitroglycerin (TNG), as well as their sediment and biomass sorption partition coefficients in selected water bodies receiving munitions wastes. The data from these studies were integrated into a computer program designed to simulate the water-receiving bodies, and concentration-time dependent curves were constructed to estimate the component concentration at various locations in the receiving water body. These curves can be used to estimate the persistence of munition compounds in the aquatic environment.

The photochemical and microbial transformation rate constants, estimated half-lives, sediment and biomass sorption partition coefficients, and volatilization rate constants for TNT, 2,4-DNT, RDX, and TNG in various water bodies appear in the summary table on the next page.

Photolysis was found to be the primary process for TNT loss in the environment. Naturally occurring substances accelerate the photochemical transformation and the photochemical rate constants are water-body dependent. Microbial transformation is slow in most natural environments (cell populations of about 10^6 cells ml^{-1}) but can become rapid when cell populations exceed 10^9 cells ml^{-1}. TNT was not used as a sole carbon source. When we used chemical loadings of the mid-1970s (Rosenblatt et al., 1973), computer simulations of the Holsten River, New River, and Waconda Bay indicated that TNT concentrations will rapidly decline to very low levels within short distances from the discharge point.

RDX is transformed slowly through photolysis, leading to half-lives of up to 14 days in the Holston River during the winter. Biotransformation did not occur under aerobic conditions common to most rivers; however, in localized anaerobic environments, biotransformation readily

SUMMARY TABLE

RATE CONSTANTS AND PARTITION COEFFICIENTS FOR ENVIRONMENTAL PROCESSES AFFECTING THE HALF-LIFE OF TNT, RDX, 2,4-DNT, AND TNG IN SELECTED WATER BODIES

Compound		Photolysis (h^{-1})	Microbial k_b[1] (h^{-1})	Microbial k_b[2] (ml $cell^{-1}$ h^{-1})	Half-life with Major Process (Days)	Sorption Partition Coefficient Sediment	Sorption Partition Coefficient Biomass	Volatilization Rate Constant (h^{-1})
TNT	Distilled water	6.1×10^{-2}	--	--	0.46 (P)	--	--	
	Waconda Bay	5.4×10^{-2}	3.6×10^{-3}	2.6×10^{-10}	0.05	--	--	
	Holston River	3.6×10^{0}	3.6×10^{-3}	2.6×10^{-10}	0.01	46	93	
	New River	3.6×10^{0}	3.6×10^{-3}	2.6×10^{-10}	0.01	--	--	
RDX	Distilled water	2.3×10^{-3}	--	--	--	--	--	
	Holston River	2.1×10^{-3}	--	--	13.8 (P)	5.5	47	
2,4-DNT	Distilled water	8.3×10^{-2}	--	--	--	--	--	
	Waconda Bay	1.3×10^{-1}	$7.2 \quad 10^{-1}$	4.7×10^{-8}	0.23 (P and B)	14	64	3.4×10^{-3}
	Holston River	2.6×10^{-1}	--	--	--	--	--	
TNG	Distilled water	0.7×10^{-6}	--	--	--	--	--	
	New River	1.4×10^{-6}	1.0×10^{-3}	1.0×10^{-9}	29 (B)	27	37	

P = Photolysis

B = Biotransformation

-- = Not determined

occurred. Computer simulations of RDX in the Holston River indicate that the RDX concentration is controlled mainly by stream dilution, and that RDX may be found beyond the Holston Army Ammunition Plant boundaries.

The loss of 2,4-dinitrotoluene (2,4-DNT) in the aqueous environment is controlled by both photolysis and biotransformation. Half-lives are estimated to range from 3 to 6 h in natural waters. Although the photolysis rates of 2,4-DNT are slower than those of TNT, similar photolytic-rate-accelerating effects were observed in natural waters. Compared to photolysis in distilled water, 2,4-DNT is readily converted to CO_2 by microorganisms that use 2,4-DNT as a growth and energy substrate. Computer simulations of the New River and Waconda Bay indicate that 2,4-DNT will approach very low concentrations under mid-1970s loading and mean flow conditions within short distances from the discharge point.

Trinitroglycerin (TNG) was found to biotransform readily with high cell populations (10^9 cells ml^{-1}), yielding a half-life of 1 h. In the New River, where microbial populations are estimated to be 10^6 cells ml^{-1}, the half-life was 29 days. Photolysis was also slow, yielding a half-life of 60 days in the New River. Thus, in the absence of high cell populations, TNG appears to be stable in the aquatic environment. Computer simulations of TNG in the New River indicate that TNG will be diluted where the waste stream meets the New River and remain at a constant level until further river dilutions occur. Hydrolysis of TNG is unimportant under these environmental conditions.

The sediment sorption and biosorption partition coefficients for TNT, RDX, 2,4-DNT, and TNG are extremely low, indicating that sorption will not be a significant process for removing these compounds from the aqueous environment.

Volatilization is not a significant process for any of the chemicals investigated.

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I INTRODUCTION

The U.S. Army is developing a scientific data base to assess the human health and environmental hazards of munition-related water pollutants. This data base consists of toxicological effect data in both mammalian and aquatic species and environmental fate data from which the persistence of the chemical in the environment can be estimated. From these data, the Army can assess the hazards associated with these wastewaters and take appropriate ameliorative actions.

The pollutants for which the data base is being developed are 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2,4-dinitrotoluene (2,4-DNT), and trinitroglycerin (TNG). While the toxicological data are being obtained elsewhere, this study was undertaken to identify the transport and transformation processes that affect the persistence of these compounds in the aquatic environment. In Phase I, a literature review was performed to determine the processes that transform or remove the above chemicals in the environment (Spanggord, et al., 1980). In Phase II, laboratory studies were performed to investigate the importance of biotransformation, photolysis, sorption, and volatilization as fate processes, and detailed kinetic investigations were performed to obtain rate constants and half-lives for these processes in both distilled and natural waters.

Our investigation is based on the now well-accepted ideas that the fate (loss and movement) of a chemical in the environment can be expressed as the sum of all physical, biological, and chemical processes acting independently on the chemical and that in many cases these processes can be formulated as second-order kinetic or equilibrium processes (Smith, et al, 1978; Mill, 1980). Thus, for a single kinetic process the rate of loss follows the relation:

$$R_n = k_n(C)(E)_n \quad , \tag{1}$$

where k_n is the second-order rate or equilibrium constant, and E_n is the environmental component (such as acid, micro-organisms, or photons) acting on the chemical C, (C, k, and E are in units to give R in moles liter^{-1} second^{-1}). If we assume that for the interval of our measurement, E_n is constant, then the second-order process becomes pseudo-first-order

$$R_n = k'_n(C) \quad , \tag{2}$$

where $k'_n = k_n(E)$. The total rate of loss of the chemical is then:

$$R_{nT} = \Sigma k'_n(C) \quad , \tag{3}$$

and the net half-life of the chemical is

$$t_{\frac{1}{2}} = \ln 2/\Sigma k'_n \quad . \tag{4}$$

In previous studies we and others have shown that only a few environmental processes are important in controlling loss or movement of chemicals in aquatic systems. These processes include sorption to sediment, volatilization, oxidation, hydrolysis, photolysis, and microbiological transformation (Smith et al., 1978; Mill, 1980). In Phase I we showed that oxidation, reduction, and volatilization would most likely be unimportant for the munition chemicals selected by the Army for investigation. Therefore, in Phase II we focused our attention on sorption, photolysis, hydrolysis (TNG), and microbial transformation.

Figure 1 illustrates the overall technical approach, beginning with selection of the chemicals. In most cases, the information in the literature on these compounds was insufficient to allow us to decide which of the environmental processes was most important for detailed

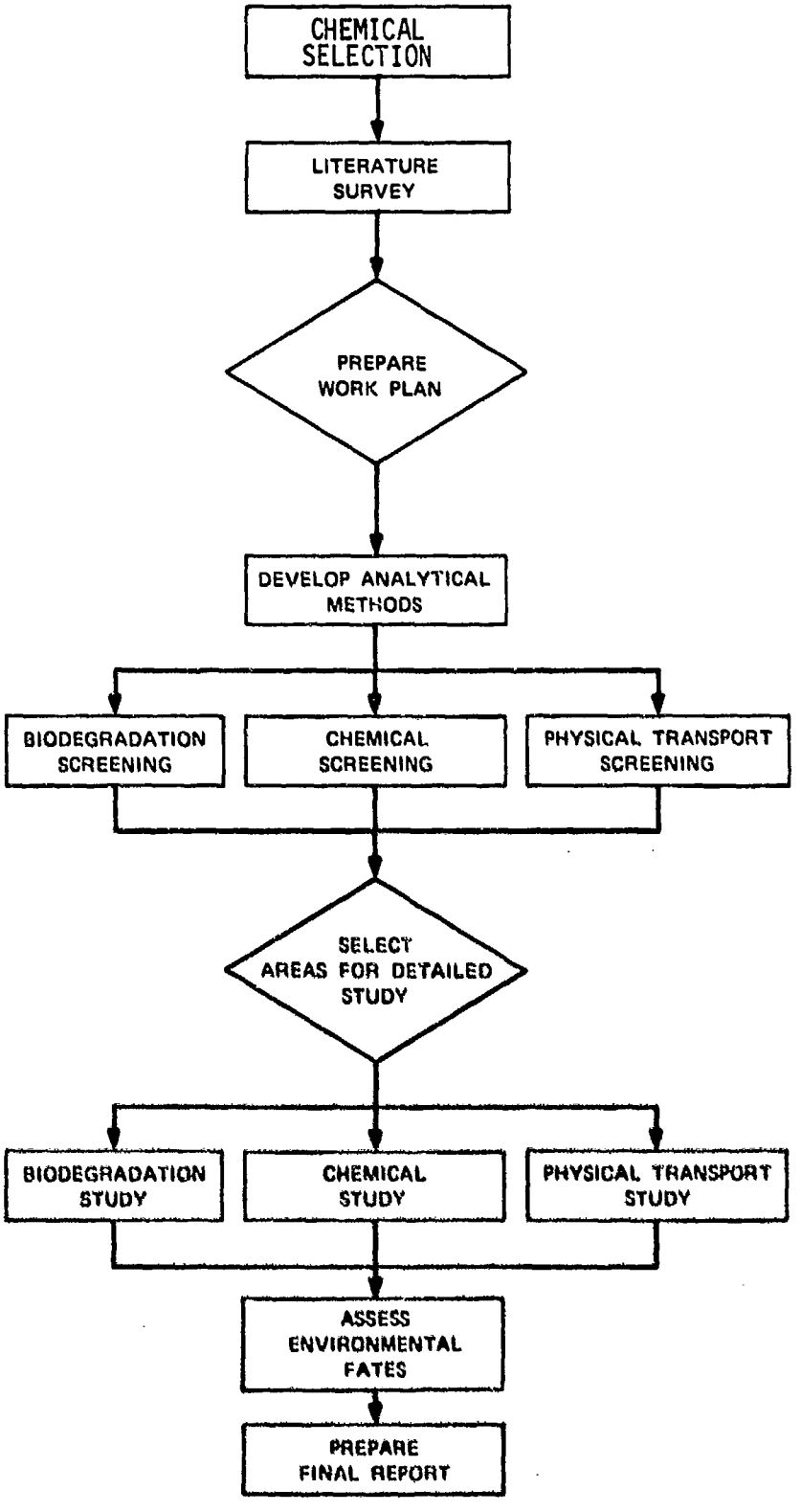


FIGURE 1 FLOWCHART FOR TECHNICAL APPROACH

study. Therefore, screening studies were conducted to estimate the relative importance of each process. Pathways that appeared to be most important were studied in detail to obtain rates and to identify products. To maximize the amount of relevant data produced and minimize the cost, processes that did not appear to be significant were not carried past the screening stage.

After all the important rate constants were measured for a chemical, its environmental fate was assessed using a multicompartment computer model developed at SRI under a previous contract (Smith et al., 1978). With the model we estimated the probable concentrations and distribution of a specific munitions chemical resulting from low-load continuous discharge of the chemical into a specific receiving-water body.

This model was programmed to simulate the hydraulic and hydrological properties of the New River in Radford, Virginia (Radford Army Ammunition Plant); the Holston River in Kingsport, Tennessee (Holston Army Ammunition Plant); and Waconda Bay of the Tennessee River near Chattanooga, Tennessee (Volunteer Army Ammunition Plant). These sites are or were the primary receiving bodies for munitions discharge and represent critical areas where water quality criteria need to be established and pollution abatement requirements need to be estimated. Laboratory studies provided the key rate constants, k_n, for the model. These rate constants, together with environmental parameters (E_n), provide the basis for estimating concentrations and distributions of a chemical as a function of time and distance from the source.

This information will allow the Army to develop an optimum strategy for pollutant abatement for these sites and will assist in the overall hazard assessment of munition-related water pollutants.

II OBJECTIVES

The objectives of this study were to:

(1) Identify the important transformation and transport processes that affect TNT, RDX, 2,4-DNT, and TNG in selected water bodies.

(2) Determine the rate constants and partition coefficients for the important processes through laboratory studies.

(3) Simulate by computer modeling the movement and losses of each compound in water bodies having hydrologic and environmental parameters similar to the Holston River, New River, and Waconda Bay.

III BACKGROUND AND METHODS

The environmental assessment contained in this report is based on the assumption that the fate of the studied chemicals can be estimated from the rate constants of the transport and transformation processes leading to the loss of the chemical from the aquatic environment. The following sections describe briefly (1) the mathematical development of the rate constants used for this assessment and (2) the simulation model requirements for the sites in this investigation. The experimental methods are described in detail in Appendix A, and the input parameters for the SRI model are detailed in Appendix B.

A. Photochemical Transformation Rate

Chemicals undergo photochemical transformation in the aquatic environment through the absorption of photons at wavelengths of 290 nm and above. These wavelengths represent the solar spectrum penetrating the earth's upper atmosphere and reaching the surface.

The rate of absorption of light, I_A, by a chemical, C, at a particular wavelength in dilute solution in pure water can be determined by the following equation (Zepp and Cline, 1977)

$$I_A = 2.3r\epsilon I_\lambda [C] = k_a [C] \qquad (5)$$

where

ϵ = molar absorptivity in liters $cm^{-1} mole^{-1}$

I_λ = a light intensity term in Einsteins $liter^{-1} s^{-1}$

[C] = chemical concentration in moles $liter^{-1}$

k_a = rate constant for light absorption

r = a correction term for water depth.

If the quantum yield, ϕ, represents the fraction of absorbed light leading to a chemical reaction, then the rate of chemical loss can be expressed as follows:

$$-\frac{dC}{dt} = I_A\phi = \phi k_a[C] = k_p[C] \qquad (6)$$

where (at a single wavelength)

$$k_p = 2.3r\varepsilon\phi I_\lambda \quad . \qquad (7)$$

Integration of Eq. 6 gives

$$\ln \frac{C_o}{C_t} = k_p t \quad , \qquad (8)$$

where C_o and C_t are concentrations of the chemical at time zero and at time t. This equation allows us to determine the rate constant for photolysis at a single wavelength from a regression fit of ln C_t as a function of time, in which k_p is the slope of this first-order plot. Once k_p is known, the quantum yield, ϕ, is obtained from equation 7, for which the molar absorptivity is measured from the absorption spectrum at the studied wavelength and the light intensity is obtained by actinometry.

Since the quantum yield, ϕ, in general, does not vary significantly with wavelength, the photolysis rate constant in sunlight, $k_p(s)$, can be represented (Eq. 9) as the quantum yield times the summation of the molar absorptivity times the light intensity over the solar spectral region:

$$k_{p(s)} = \phi\Sigma\varepsilon_\lambda I_\lambda \quad , \qquad (9)$$

and the half-life in sunlight is

$$t_{\frac{1}{2}(s)} = \frac{\ln 2}{k_{p(s)}} \quad . \qquad (10)$$

Values for ε_λ are obtained from laboratory measurements, while values for the sunlight intensity I_λ, are obtained from the literature for a particular time of day, season, and latitude. The calculated rate constant, $k_{p(s)}$, can be compared to the measured value, $k_{p(m)}$, obtained in pure water in sunlight using equation 8. In our experience, the calculated and measured rate constants obtained by the above procedures give excellent agreement, usually within a factor of two.

B. Biotransformation Rate

In natural waters many types of microorganisms are found; the populations may vary with the type of water body, season, and organic substrates being introduced. In this study, we attempted to obtain microorganisms biotransforming specific chemicals by enrichment procedures using water from the water bodies receiving the munition chemicals of interest. Once an enrichment culture was developed, studies were performed with a specific chemical to determine the biotransformation rate constant.

The biotransformation rate is a function of biomass and chemical concentration. When an organic chemical is utilized by microorganisms as the sole carbon source and its concentration limits the rate of microbial growth, the relationship between the chemical utilization rate and the chemical concentration can be expressed by the Monod kinetic equation as:

$$-\frac{dC}{dt} = \frac{\mu[X]}{Y} = \frac{\mu_m}{Y} \cdot \frac{[C][X]}{K_c+[C]} = k_b \frac{[C][X]}{K_c+[C]} \quad , \qquad (11)$$

where [X] is the biomass per unit volume, μ is the specific growth rate, μ_m is the maximum specific growth rate, [C] is the concentration of growth limiting chemical, K_c is the concentration of chemical supporting the half-maximum specific growth rate (0.5 μ_m), Y is the cell yield, and k_b is the substrate utilization constant and equal to μ_m/Y.

The parameters μ_m, Y, and K_c can be evaluated by performing a series of experiments with different chemical concentrations and low-level inocula or with continuous cultures (Smith et al., 1978). However, these methods are time-consuming, and large amounts of chemical are required.

For many pollutants in the environment, chemical concentrations are very low and $C << K_c$. Under these conditions, equation 11 reduces to equation 12, which is a simple second-order relation:

$$-\frac{dC}{dt} = k_b \frac{[C][X]}{K_c} = k_{b2}[C][X] \quad . \tag{12}$$

Furthermore, if the rate study is conducted using a large microbial population and a low chemical concentration, the bacterial cell count will remain constant under the experimental conditions because the cell growth will not be affected greatly by the consumption of the test chemical. The biotransformation rate is then assumed to be a pseudo-first-order rate process with respect to the chemical, C, and is described by equation 13:

$$-\frac{dC}{dt} = k_b'[C] \quad , \tag{13}$$

where k_b' is a pseudo-first-order rate constant. Integration of equation 13 yields equation 14:

$$\ln \frac{C_o}{C_t} = k_b' t \quad , \tag{14}$$

where C_o and C_t are concentrations of chemical at time zero and t. By plotting ln C as a function of time, k_b' can be determined as the slope of the line.

Equation 12 shows that the rate of transformation is linearly related to the microbial population. Therefore, if the rate constant k_b' is measured with constant values of [X], the value of k_{b2} can be estimated from the relation (equation 15):

$$k_{b^2} = \frac{k_b'}{[X]} \quad , \tag{15}$$

where k_{b^2} is the second-order rate constant and where [X] is estimated from microbial plate counts.

C. Volatilization Rate

The volatilization of a chemical from a liquid to a gas phase can be controlled by transfer across a liquid-phase boundary layer, a gas-phase boundary layer, or both. The volatilization rate constant, k_v, is expressed in terms of the mass transfer rates of the substance across liquid- and gas-phase boundary layers. The general expression for k_v is:

$$k_v = \frac{1}{L}\left(\frac{1}{k_\ell} + \frac{RT}{H_c k_g}\right)^{-1} \quad , \tag{16}$$

where:

k_v = Volatilization rate constant (h^{-1})

L = Depth (cm), which equals the liquid volume, V, divided by the interfacial area, A

k_ℓ = Liquid film mass-transfer coefficient (cm h^{-1})

R = Gas constant (liter torr K^{-1} $mole^{-1}$)

T = Temperature (K)

H_c = Henry's law constant (torr liter $mole^{-1}$)

k_g = Gas film mass-transfer coefficient (cm h^{-1})

The relative magnitude of the two terms in equation 16 determines whether liquid- or gas-phase mass transfer controls the volatilization.

If H_c > 3500 torr M^{-1}, then mass transfer in the liquid phase controls about 95% of the volatilization rate constant. If 10 < H_c < 3500 torr M^{-1}, then mass transfer in both the liquid and gas phases are significant. Mass transfer is only significant in the gas phase if H_c < 10 torr M^{-1} (for a more detailed explanation see Smith et al., 1980).

Values for H_c of the munitions chemicals were estimated and are summarized in Table 1.

Table 1

H_c OF MUNITIONS CHEMICALS

Chemical	Estimated Value of H_c (torr M^{-1})
TNT	0.18
RDX	2×10^{-3}
TNG	> 0.06
2,4-DNT	3.4

The estimated values of H_c suggest that the volatilization rate of the four munitions chemicals will be mostly gas phase mass transport resistance limited. If that is true, then the first term in equation 16 is negligible and equation 16 reduces to

$$k_v^C = \frac{H_c k_g^C}{LRT} \quad , \tag{17}$$

where the added superscripts indicate the chemical.

If H_c is known and if k_g^C and L can be estimated for a specific water body, the value of k_v^C in the environment can be calculated. We have proposed (Smith et al., 1980) that the ratio of the gas-phase mass-transfer coefficients of two chemicals should be equal to the ratio of the diffusion constants in air. Furthermore, a convenient choice of the second chemical is water, because the evaporation rate of water (or any pure chemical) must be 100% controlled by gas-phase mass transfer (Liss and Slater, 1974). Therefore,

$$\frac{k_g^C}{k_g^W} = \left[\frac{D_g^C}{D_g^W}\right]^m = B = c \quad , \tag{18}$$

where D_ℓ and D_g are the liquid- and gas-phase diffusion coefficients, respectively, and the superscripts C and W are chemical and water, respectively. Then, rearranging equation 18 and substituting in equation 17 yields equation 19

$$k_v^C = \frac{H_c \left[\frac{d_g^C}{D_g^W}\right]^m k_g^W}{LRT} \quad . \tag{19}$$

Thus, k_v^C is proportional to k_g^W for each volatilization rate measurement.

The value of k_g^W can be calculated from the water evaporation rate by equation 20

$$N^W = k_g^W \frac{\left[P_s^W - p^W\right]}{RT} \quad , \tag{20}$$

where N^W is the water evaporation flux in moles cm^{-2} h^{-1}, and P_s^W and p^W are the saturated partial pressure and partial pressure of water, respectively, in torr. Thus, k_g^W can easily be determined for a specific laboratory experiment or natural water body by measuring the water evaporation rate and the wet and dry bulb temperatures.

D. Sorption Studies

The principal measurement of interest in solid-liquid sorption is the amount of solute adsorbed when a known amount of a solution of known concentration is equilibrated with a known amount of solid. A plot of the amount of solute adsorbed per unit mass of solid (C_s) against the equilibrium concentration of the solution at a constant

temperature (C_w) is called a sorption isotherm. The experimental data for sorption of an organic compound on a natural soil or sediment usually fit the Freundlich isotherm, which is the empirical relationship (Hamaker and Thompson, 1972) shown in equation 21

$$C_s = K\left[C_w\right]^{1/n} \quad , \tag{21}$$

where K and n are constants. At equilibrium and at low adsorbate concentrations, n is often nearly equal to 1. If n equals 1 and C_s and C_w are expressed in the same units, such as $\mu g\ g^{-1}$, then K is a partition or distribution constant defined by equation 22:

$$C_s = K_p C_w \quad . \tag{22}$$

Several studies have shown that equation 22 is obeyed for sorption on natural sediments from very dilute solutions of neutral organics in water (Smith et al., 1977; Karickhoff et al., 1979; Chiou et al., 1979). Moreover, because there is evidence (e.g., Lambert, 1968) that neutral organic species predominantly sorb on the organic content of sediments, it has become common practice to normalize adsorption coefficients for the fraction of organic carbon in the sediment (Karickhoff et al., 1979). Thus, if equation 22 is obeyed and the fraction of organic carbon is F_{oc}, then

$$K_{oc} = \frac{K_p}{F_{oc}} \quad , \tag{23}$$

where K_{oc}, the sorption partition coefficient normalized for organic carbon content, has the units of milliliters of solution per gram of organic carbon.

The assumed linear relationship of K_p with F_{oc} in equation 23 implies that (1) the density of available sorption sites on the organic matter is independent of F_{oc}, and (2) sorption on the clay fraction of the sediment is small.

If equation 22 is valid, the calculated values of K_{oc} may be used to predict the partitioning of a chemical between water and any sediment with a known organic carbon content. Furthermore, solubility can be correlated with K_{oc} values, using an expression of the form:

$$\log K_{oc} = a \log x_s + b \quad , \tag{24}$$

where x_s is the mole fraction solubility, and a and b are correlation constants. Equation 23 gives good correlations of K_{oc} and x_s (Chiou et al., 1979; Karickhoff et al., 1979; Kenaga and Goring, 1978) and thus may be used to verify the reliability of solubility and sorption measurements.

For the work reported here the data shown in Figure 2 were used to estimate values of K_{oc}. The least-squares fit of this data is

$$\log K_{oc} = -0.27 - 0.732 \log (C_{sol}) \quad , \tag{24a}$$

where the solubility of the chemical in distilled water, C_{sol}, is expressed in M.

To measure the sorption isotherm, the amount of sediment and compound must be adjusted so that measurable amounts of the compound are contained in both solution and sediment after equilibrium is established. If this is not done, then the error in the measured value of K_{oc} and K_p is increased significantly. The estimated value of K_{oc} was calculated from the literature values of the solubilities and is reported in Table 2.

The procedure and equations used to calculate the appropriate concentrations of chemical and sediment for the isotherm measurements are described in detail by Smith and Bomberger (1980).

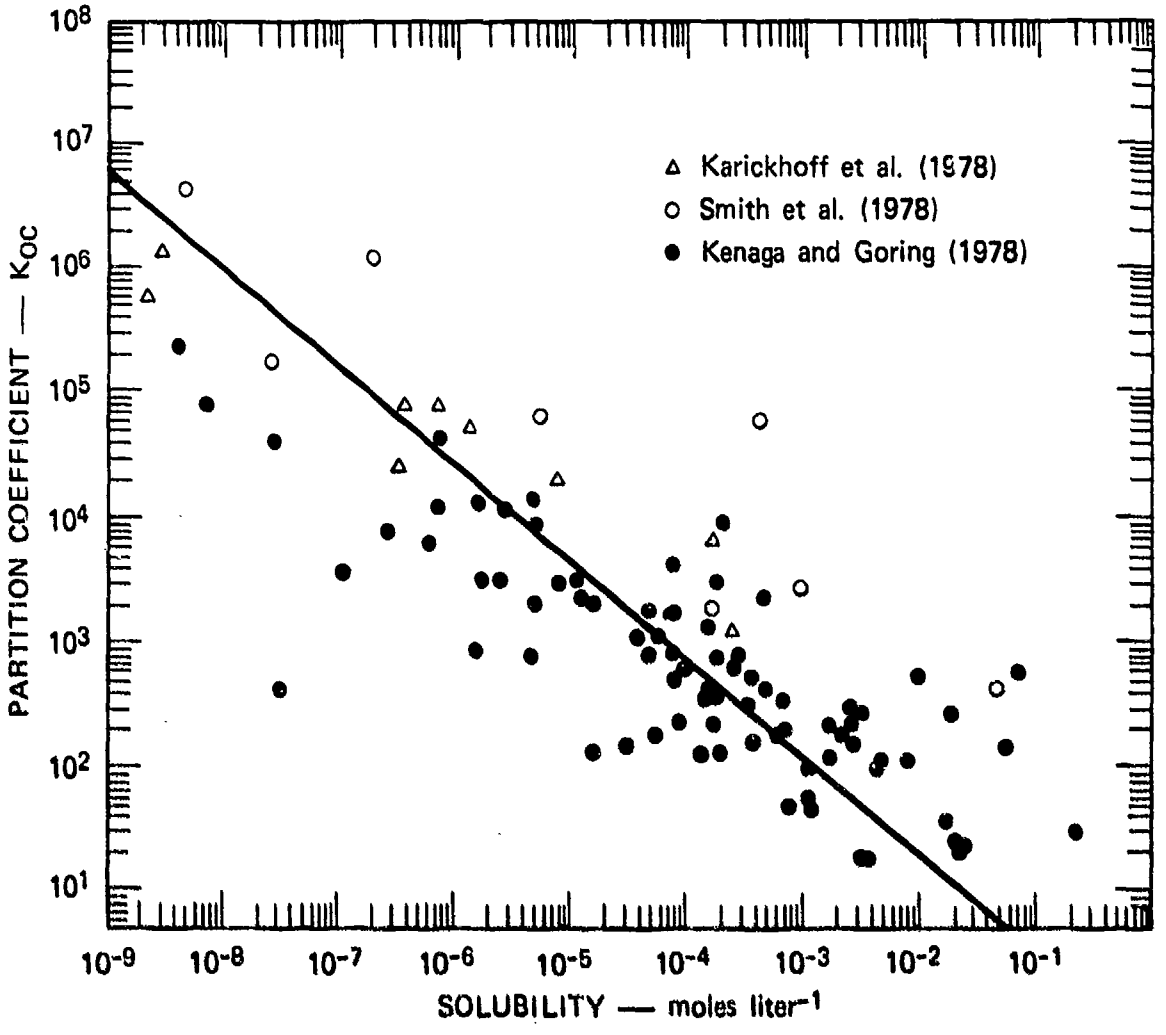


FIGURE 2 SOIL OR SEDIMENT PARTITION COEFFICIENT OF CHEMICALS AS A FUNCTION OF SOLUBILITY IN WATER

Table 2

PREDICTED VALUES OF K_{oc}

Chemical	Solubility (Moles liter^{-1})	Predicted K_{oc}
TNT	5.4×10^{-4}	130
RDX	2.0×10^{-4}	270
2,4-DNT	1.5×10^{-3}	63
TNG	5.3×10^{-3}	25

Batch sorption isotherm measurements were used to determine the value of K_p for TNT, TNG, 2,4-DNT, and RDX. The general design is shown in Table 3.

Table 3

EXPERIMENTAL PLAN FOR THE BATCH ADSORPTION ISOTHERM MEASUREMENTS

Concentration of Chemical	Number of Flasks	
	No Sediment	With Sediment
None	1	1
Low	1	2
High	1	2

Control flasks with no sediment were used to determine the initial concentration of each component. The blank containing sediment but no compound was used to check for interfering substances that either leached from the suspended sediment or were extracted during analysis of the sediment. Screening isotherms were performed only at one chemical concentration.

After the equilibration time of 160 h, the contents of each flask were centrifuged in a high-speed centrifuge. The supernatant was carefully decanted into another vessel, and both the supernatant and the sediment were analyzed.

E. Simulation Model

The simulation model used in this program was described by Smith et al., (1977) and from here on will be denoted as the SRI Model.

The function of the SRI Model is to simulate the hydraulic and hydrologic conditions of the water-receiving bodies into which wastes are discharged and to estimate the probable concentrations of pollutants as a function of time in the receiving water body. Determination of pollutant concentrations is based on the relative importance of the sorption equilibrium and the rates of photolysis, hydrolysis, oxidation, biotransformation, and volatilization on removing the pollutant from the aquatic system. The rate and equilibrium constants were determined from laboratory studies using the methods described by Smith et al. (1977) and are summarized in the following sections. These rate and equilibrium constants were then extrapolated to natural water environments and used as input parameters for the model.

In the SRI Model, the receiving water body is divided into compartments that are connected by interflows (or stream flows). Each compartment is assumed to be a homogeneous, constant-volume-mixing reactor where the pollutants undergo various transformation processes at rates determined by the environmental conditions of that compartment. Figure 3 shows diagrams of a compartmented pond, river, and lake and gives a visual indication of the compartmentalization. The number of compartments used to describe a receiving water body is selected by the investigator.

Three water bodies that receive munition wastes were simulated. The Holston River receives wastes from the Holston Army Ammunition Plant (AAP), where RDX is manufactured and TNT from shell-loading

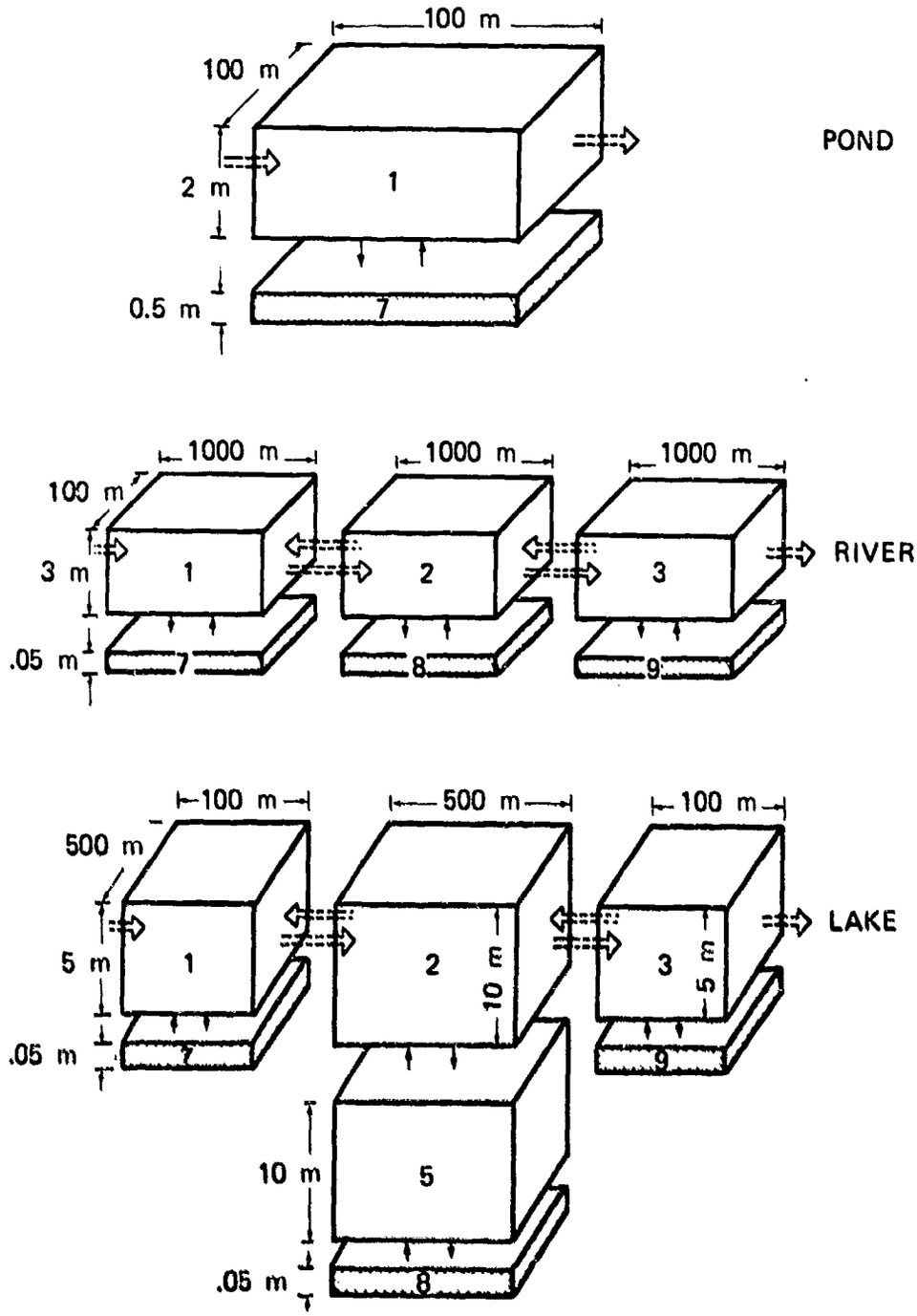


FIGURE 3 PHYSICAL CONFIGURATIONS OF THE POND, RIVER, AND LAKE SIMULATIONS

operations enters the waste stream. The New River receives wastes from Radford AAP where TNT and TNG are manufactured, and 2,4-DNT enters the waste stream from propellant operations. Waconda Bay receives wastes from Volunteer AAP, where TNT is manufactured; 2,4-DNT enters the waste stream as the primary component of condensate water resulting from the purification of TNT. The receiving water bodies, their location, the munition chemicals investigated in the water body, the length of the water body studied, and the number of compartments used to describe this length appear in Table 4.

Table 4

WATER BODY, REACH, COMPARTMENTS, AND MUNITIONS INVESTIGATED IN EACH RECEIVING WATER BODY

Receiving Water Body	Location	Munition Chemicals Investigated	Length of Studied Reach (km)	Number Compartments
Holston River	Kingsport, TN	TNT, RDX	8.4	21
New River	Radford, VA	TNT, TNG 2,4-DNT	14	28
Waconda Bay	Chattanooga, TN	TNT, 2,4-DNT	4.8	12

Table 5 shows those munition chemicals produced and used in the various facilities. Because some chemicals were not produced in the plants, but were used there, the model simulations included both chemicals produced and those used.

Table 5

MUNITION CHEMICALS PRODUCED AND USED IN VARIOUS FACILITIES

Facility	Produced				Used			
	TNT	2,4-DNT	RDX	TNG	TNT	2,4-DNT	RDX	TNG
Holston AAP			x		x		x	
Radford AAP	x			x	x	x	x	x
Volunteer AAP	x				x	x[a]		

[a]Major component of condensate water discharged.

The SRI Model requires data on the physical dimensions of the compartments (size), hydrologic data of the area being simulated, chemical loading data, and rate constants for the transport and transformation processes. Compartment dimensions were estimated by multiplying the cross-sectional areas of the water bodies times the length of each compartment. Hydrologic data were obtained from published sources, including the U.S. Geological Survey, and chemical loading data were obtained from published military reports. The rate constants were obtained from the results of the work described in this report.

Once these data were entered into the model program, computations were performed to determine the mass changes in each compartment due to transformation processes, the mass changes in the aqueous and solid phases due to sorption or volatilization, and the new masses in each compartment from the assumed inflow-outflow conditions. From these computations, the model output described the distribution of mass and concentration of the chemical under investigation with respect to time (or distance) from the discharge point. From these distributions of chemical, concentration-time profiles were developed that allow one to predict the chemical concentration at various locations in the receiving water body and to estimate the persistence of the chemical for the given loading rate and hydrologic conditions.

Detailed site descriptions for the AAPs in this study and the water quality parameters selected for each site are described in Appendix B. The results of the SRI Model application are described in the Results and Discussion subsection for each compound.

IV RESULTS AND DISCUSSION

A. 2,4,6-Trinitrotoluene (TNT)

1. Background

The Phase I literature review (Spanggord et al., 1980) indicated that photolysis would be a significant fate process for TNT, based primarily on the work of Burlinson (Burlinson and Glover, 1976; Burlinson, 1978) with natural waters. Biotransformation was also considered to be important because numerous organisms apparently can transform TNT. Although sorption was not considered to be significant, the lack of reliable literature data suggested that further studies were needed to define the partitioning processes for TNT. Therefore, detailed photochemical, microbial, and sorption-partitioning studies were conducted.

2. Photochemistry

Photolysis studies of TNT in air-saturated distilled water were complicated by the finding that the products of TNT photolysis accelerated the photolysis rate of TNT. This acceleration in rate is shown by the upper curve in Figure 4, which shows that the photolysis rate of a 0.11-ppm solution of TNT at 313 nm increased substantially after approximately 50% loss of the TNT. A similar acceleration in photolysis rate was found in another experiment when a 1.1 ppm solution of TNT was photolyzed for over 2 TNT half-lives, with more than 20 values from various time points taken during the experiment. Because only distilled water and highly purified TNT were used in these experiments, the rate acceleration must be due to products formed during photolysis. This conclusion is supported by an experiment in which additional TNT was added to a previously photolyzed TNT solution. Subsequent photolysis of this solution resulted in an initially rapid rate of TNT loss that was similar to the rate observed at the end of the original photolysis

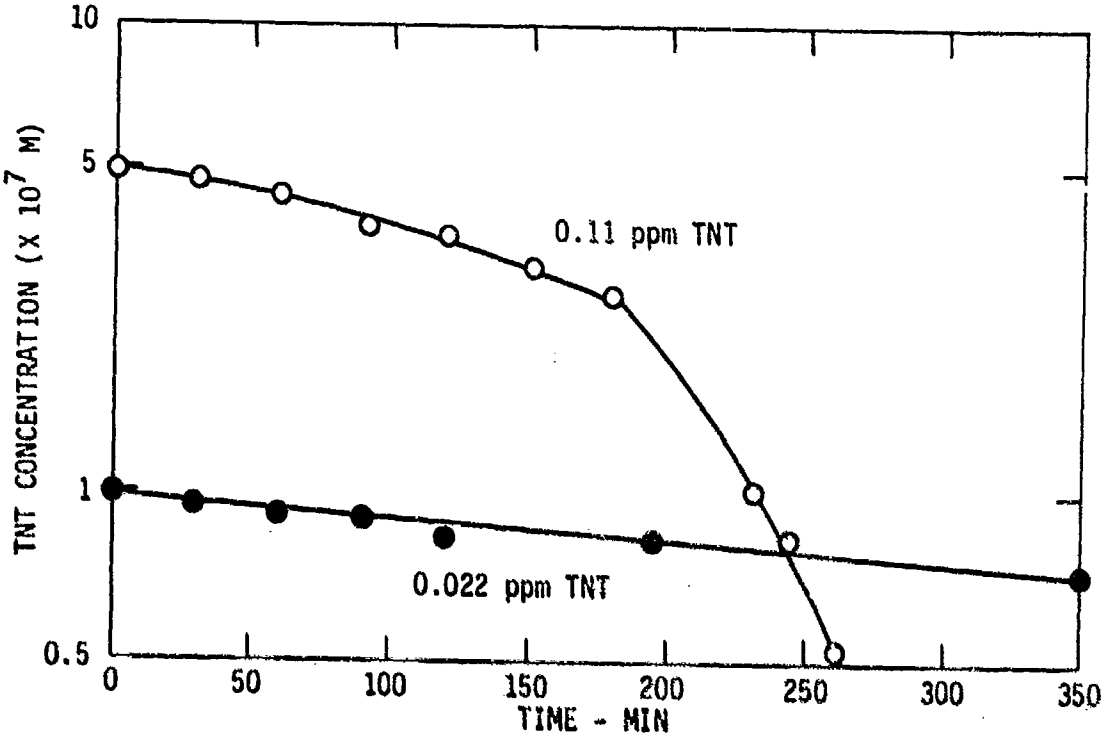


FIGURE 4 PHOTOLYSIS OF TNT IN PURE WATER AT 313 NM

experiment. As shown in Figure 4, when the initial concentration of TNT was lowered to 0.022 ppm, the photolysis of TNT showed good first-order kinetic behavior, which remained linear for over 700 minutes of photolysis time; presumably the low concentration of TNT product(s) in this experiment is insufficient to accelerate the TNT photolysis. The photolysis rate constant from this latter experiment is the value given in Table 6 for the air-saturated, distilled-water TNT solution photolyzed at 313 nm. The other rate constants in Table 6 for 313-nm and sunlight photolyses, were calculated using the low conversion rates of TNT (before the distinct downward curvature is apparent).

The data in Table 6 show that TNT in the argon-purged (nonsaturated) aqueous solutions photolyzes approximately three times faster than TNT in the air-saturated solutions. As discussed later in this section, this may be because oxygen quenches triplet-excited states in photochemical reactions (Calvert and Pitts, 1967). Since aquatic systems in which chemicals are photolyzed are probably air-saturated or nearly-air-saturated, rate constants in the air-saturated water are the values of interest for environmental assessment. The sunlight photolysis rate constants were calculated for experiments of several hours duration conducted on a single day, and therefore the rate averaged over the entire day will be about one-half those listed in Table 6 if we assume approximately 12 hours of sunlight a day.

Using the rate constant for photolysis of TNT in the air-saturated water at 313 nm, we calculated the reaction quantum yield to be 2.7×10^{-3}. The same quantum yield was found for the photolysis of TNT at 366 nm, and therefore it is reasonable to assume that the reaction quantum yield of TNT will be constant over the entire solar-absorption spectrum of TNT. This value is also in fair agreement with a reported TNT quantum yield of about 1×10^{-3} (Sanders and Slagg, 1972). The UV-visible light absorption spectrum of TNT in 10% acetonitrile-90% water was also measured, and the absorption coefficients for TNT are given in Table 7.

Table 6

PHOTOLYSIS RATE CONSTANTS FOR TNT IN PURE WATER

Light Source	Air Saturated?[a]	Rate Constant k_p, $s^{-1} \times 10^5$
313 nm	Yes	3.9 ± 0.2
313 nm	No	10 ± 1
Sunlight	Yes	1.7[b] ± 0.2
Sunlight	No	5.2[c]

[a]TNT concentration in air-saturated water experiment at 313 nm was 0.022 ppm; TNT concentration in other three experiments was 1.1 ppm; 1 ppm TNT = 4.65×10^{-6}M.

[b]Half-life calculated from this rate constant is 11 hours; assuming 12 hours of sunlight per day, the half-life averaged over a 24-hour day would be approximately 22 hours.

[c]One data point.

Table 7

ABSORPTION SPECTRUM OF TNT[a] IN 10% ACETONITRILE-90% WATER

Wavelength	Absorption Coefficient (M^{-1} cm^{-1})
297.5	1800
300.0	1600
302.5	1500
305.0	1400
307.5	1300
310.0	1200
312.5	1100
315.0	1000
317.5	900
320.0	840
323.1	800
330.0	760
340.0	620
350.0	600
360.0	420
370.0	240
380.0	160
390.0	60
400.0	0

[a]TNT concentration was 4.93×10^{-6} M (1.1 ppm).

TNT was also photolyzed in filter-sterilized natural water samples from three sources: Holston River, Tennessee; Waconda Bay, Tennessee; and Searsville Pond at Stanford, California. The first two natural waters are near munition production plants and probably contain some munition byproducts or transformation products. Searsville Pond is a eutrophic pond high in humic and fulvic acid material, but free of any TNT or other munitions-related contamination. The rate constants for the 313-nm and sunlight photolyses of a 1.1-ppm TNT solution in each of these waters are given in Table 8.

Table 8

PHOTOLYSIS RATE CONSTANTS FOR TNT IN NATURAL AND DISTILLED WATERS[a]

Water	313-nm Photolysis Rate Constant $\times 10^3$, k_p (s^{-1})	Sunlight Photolysis Rate Constant $\times 10^3$, k_p (s^{-1})
Holston River	50 ± 1	120 ± 2
Waconda Bay	12 ± 1	15 ± 1
Searsville Pond	30 ± 1	58 ± 2
Distilled water	1.3 ± 0.2	1.7 ± 0.2

[a]Rate constants calculated from data taken during experiments of less than 3 hours, and are therefore not based on a 24-hour day. If we assume 12 hours of sunlight per day, the rate constants would be about one-half the stated value for a 24-hour day.

The photolyses in Holston River and Searsville Pond samples gave good first-order TNT rate-loss plots, while that in the Waconda Bay sample showed a slight acceleration in TNT loss with time (Figure 5). In sunlight in natural waters, TNT photolyzed 10 to 100 times more rapidly than in distilled water. Although it is evident from the experiments in distilled water described above that TNT photolysis products can promote the photolysis of TNT, the experiment with the

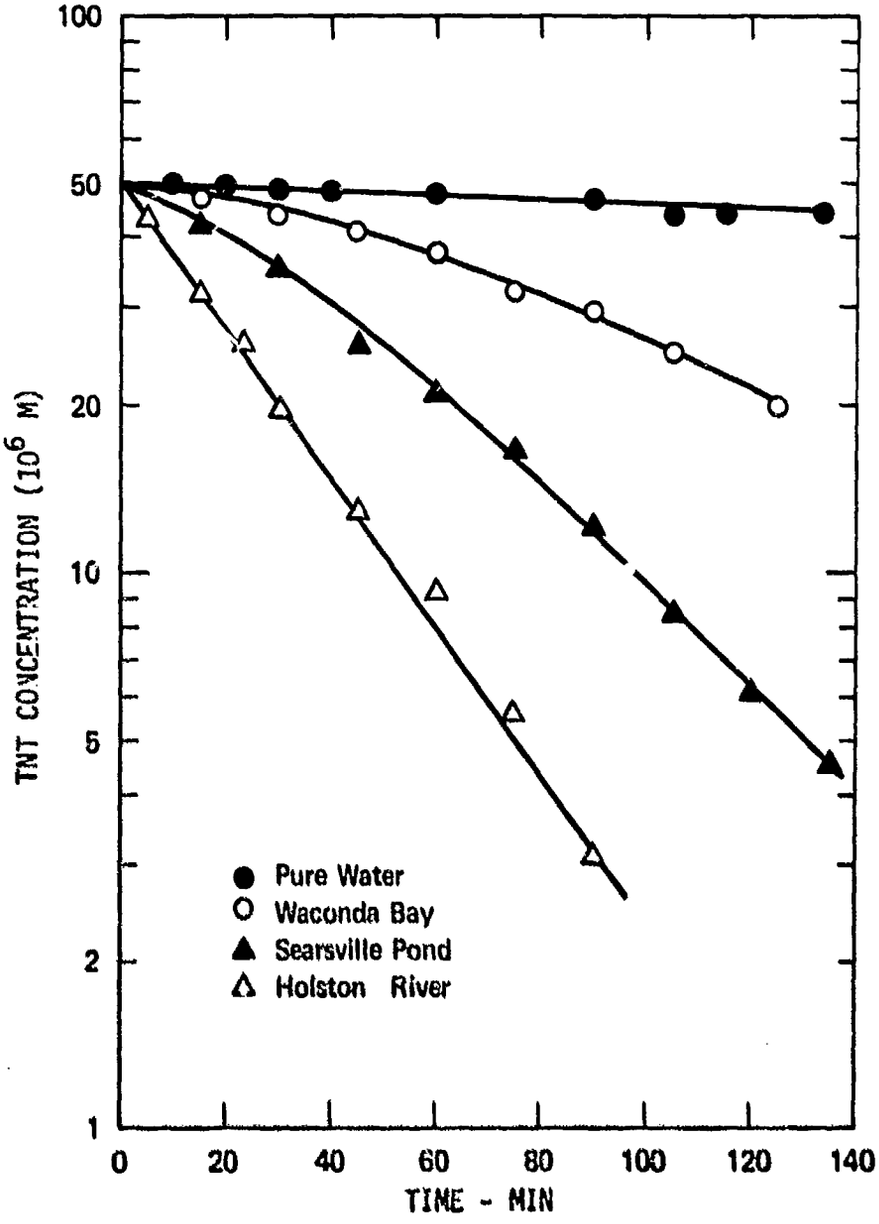


FIGURE 5 PHOTOLYSIS OF 1.1 ppm TNT IN DISTILLED AND NATURAL WATERS AT 313 NM

Searsville Pond water shows that natural substances in aquatic systems also can promote TNT photolysis. From these experiments it is not possible to evaluate how much of the photolysis rate constant in Holston River or Waconda Bay waters is caused by either the munitions transformation products present or to the natural substances present.

Because the photolysis rate for TNT was promoted both by TNT photoproducts and by natural substances, additional experiments were performed to elucidate the process(es) responsible for the accelerated photolysis of TNT. One possible indirect photolysis mechanism is that of a photosensitized reaction in which light-absorbing substances (either TNT-photolysis products or natural materials such as humic or fulvic acids) transfer excited-state energy to TNT, which then reacts.

One set of experiments was designed to determine whether the photolysis of natural organics in water could cause TNT to react. Since TNT has no measurable absorption spectrum above 400 nm, two natural waters were chosen that had absorptions tailing into the 400- to 500-nm region. The two waters used were from Searsville Pond in California and from the Aucilla River in Georgia. Solutions of 1.1 ppm TNT were photolyzed in these two waters and in distilled water (as a control) using a 750-watt tungsten lamp with a filter that cuts off all light below 420 nm. The results of these experiments are summarized in Table 9.

Table 9

PHOTOLYSIS OF 1.1 ppm TNT IN NATURAL AND DISTILLED WATER ABOVE 420 nm[a]

Water	Hours Photolyzed	TNT Lost (%)
Searsville Pond	43	28
Aucilla River	43	10
Distilled water	43	0

[a]Dark controls in all these waters showed no TNT loss.

These data show clearly that light absorption by substances in natural waters can result in reactions that transform TNT and support the suggestion that natural substances sensitize the photolysis of TNT; the quenching effect of oxygen noted earlier supports the intermediacy of a triplet-state mechanism and rules out processes involving free-radical oxidations (Mill, 1980) or singlet oxygen (Zepp et al., 1978).

Additional experiments were also conducted to determine whether TNT may be photolyzed by a triplet-sensitized mechanism. Others have suggested that the photolysis of o-nitrotoluene structures such as TNT may proceed through a triplet-state mechanism (Burlinson et al., 1979). To test this idea TNT was photolyzed in aqueous solution containing acetone, which is a known triplet-sensitizer. For these experiments, a 10-ppm TNT aqueous solution was prepared with 1.0% or 0.10% acetone. These two solutions, along with a control containing only 10 ppm TNT in water, were photolyzed simultaneously at 313 nm. The data (Table 10) show a more rapid loss of TNT in the presence of acetone, providing

Table 10

EFFECT OF ACETONE ON TNT PHOTOLYSIS[a]

TNT Concentration (ppm)	Acetone (%)	Half-life of TNT[b] (h)
10.0	0	18
10.0	0.10	9
10.0	1.0	3

[a]Photolyzed at 313 nm.

[b]Initial photolysis rate.

additional evidence for a triplet-sensitized mechanism. Additional studies must be conducted to verify the importance of this particular mechanism in natural aquatic systems and to define the kinetic

features. Once the sensitized mechanism is defined, we probably can develop the appropriate kinetic expressions for including this important process in modelling the fate of TNT in aquatic systems.

Burlinson et al. (1973) reported that the pH of an aqueous solution affected the photolysis rate of TNT, and therefore it was of interest to compare the effects of pH to the effects of a natural water on the photolysis rate of TNT. Table 11 lists the rate constants for photolysis of 1.1 ppm TNT in distilled water and in filter-sterilized water from Searsville Pond, each adjusted to pH values of 4.0 and 8.0 with minimal amounts of acid or base. From these data it is clear that the effect of

Table 11

EFFECT OF pH AND NATURAL WATERS ON PHOTOLYSIS OF 1.1 ppm TNT

Water	Rate Constant k_1, $s^{-1} \times 10^4$	
	pH 4.0	pH 8.0
Pure water	0.16 ± 0.03	0.51 ± 0.03
Searsville Pond	2.3 ± 0.1	2.5 ± 0.4

pH on the photolysis rate constant is insignificant in comparison to the effects of natural substances present in the Searsville Pond water. Probably pH has a significant effect on the photolysis rate only in waters containing very little natural substances.

In summary, TNT will be photolyzed more rapidly in natural waters than in pure water probably by a triplet-sensitized process. However, it is not possible to estimate the rate constant for the indirect photolysis of TNT in waters other than those used in this study. For environmental assessments of the Holston River or Waconda Bay, the rate constants measured in these waters should be used. The finding that TNT photolyzes more rapidly in the Searsville Pond water containing no munitions photo-products than in the Waconda Bay water shows that

natural substances may contribute more to the photolysis rate than TNT products. In view of the fact that the measured direct photolysis half-life is approximately 22 hours (based on a rate constant averaged over a 24-hour day), the direct photolysis rate constant may be regarded as a limiting photolysis rate constant for some modelling efforts to identify long-term pollution problems. The annual variation in the direct photolysis half-life for TNT has been calculated according to the procedure of Zepp and Cline (1977) using the reaction quantum yield of 2.7×10^{-3} and the absorption coefficient data in Table 7. The annual variation in half-life at 40° N latitude is shown in Figure 6, and will vary from a half-life of 14 hours in summer to 45 hours in winter; these calculated half-lives bracket the measured half-life of 22 hours in spring. Table 12 shows the variation of the direct photolysis half-life between summer and winter and as a function of latitude in the continental United States.

Table 12

SUNLIGHT PHOTOLYSIS OF TNT IN PURE WATER

Latitude (°N)	Summer Half-life (h)	Winter Half-life (h)
20	14	22
40	14	45
50	14	84

3. TNT Photoproducts

Reported photolysis products of TNT reviewed (Spanggord et al., 1980) include single aromatic ring compounds such as 1,3,5-trinitrobenzene, 4,6-dinitroanthranil, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitrobenzonitrile, and 2,4,6-trinitrobenzoic acid. The best mass balance reported for the photochemical transformation to these products accounted for less than 20% of the TNT reacted. In addition, a number

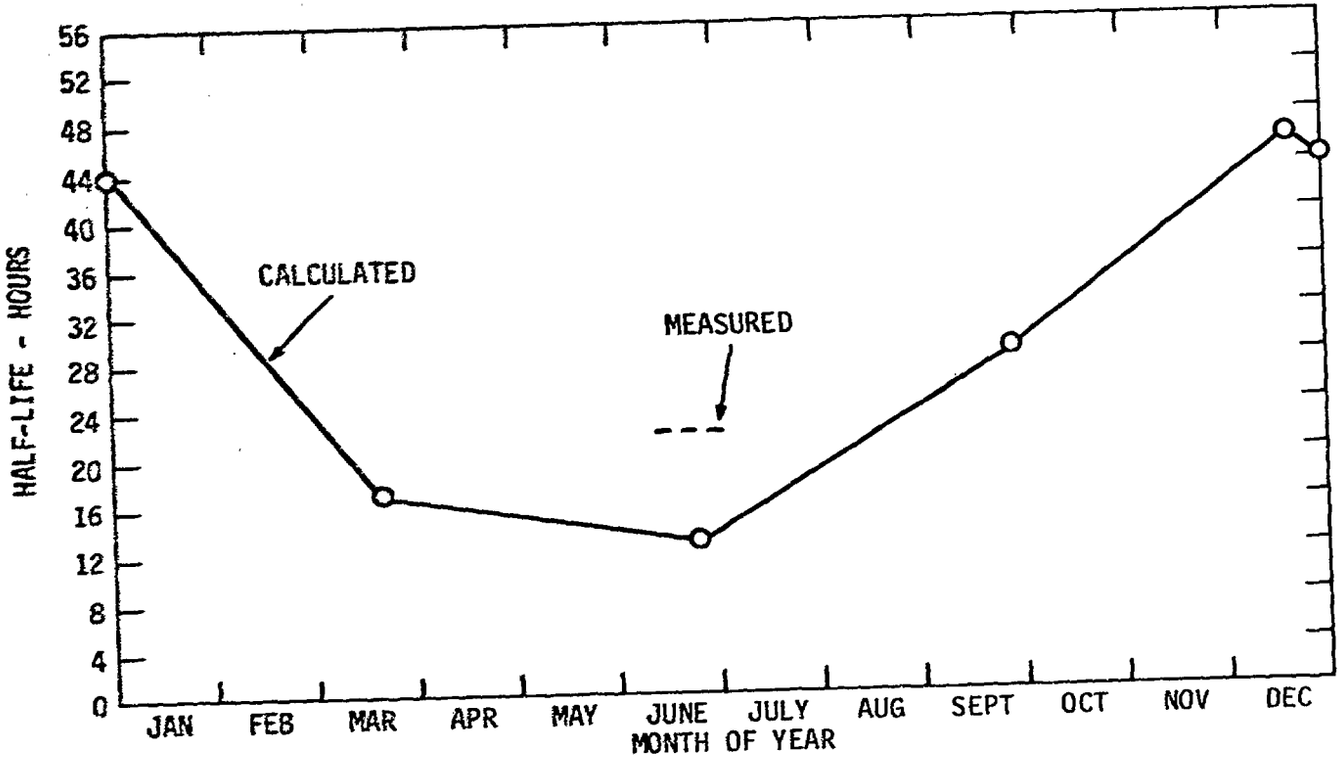


FIGURE 6 ANNUAL VARIATION OF PHOTOLYSIS HALF-LIFE OF TNT

of azo and azoxy derivatives formed by the coupling of nitroso and hydroxylamine products have been identified, and account for another 20% of the TNT loss. However, the product(s) responsible for the color in "pink water" were not previously either identified or extracted from water.

The photolysis of more than 50% of the available TNT in our studies gave most of the above products, as identified by high-performance liquid chromatography (HPLC) and silica gel thin-layer chromatography (TLC) retention times. However, when TNT was photolyzed for a short time (less than 10% TNT loss), only one major photolysis product was detected by HPLC or reverse-phase TLC (see Figure 7). This product was collected from the HPLC column using an approximately 30% acetonitrile-water mixture; a pink solution was obtained. A pink compound (PC) can be extracted directly from these solutions and purified to some extent (see analysis section).

PC collected in the above manner was found to revert thermally to TNT; no other products were detected by HPLC analysis. Rate constants for the thermal reaction were measured at 25°C and 50°C in a constant-temperature bath, and samples were removed periodically and analyzed for PC and TNT. Rate constants (k_1) were estimated from the regression line obtained from a plot of ln [PC] versus time. The data in Table 13 were obtained:

Table 13

RATE CONSTANT ESTIMATES FOR PC CONVERSION TO TNT

Temperature	k_1 (s^{-1})	$t_{1/2}$
25°C	1.01×10^{-5}	19 h
50°C	2.22×10^{-4}	52 min

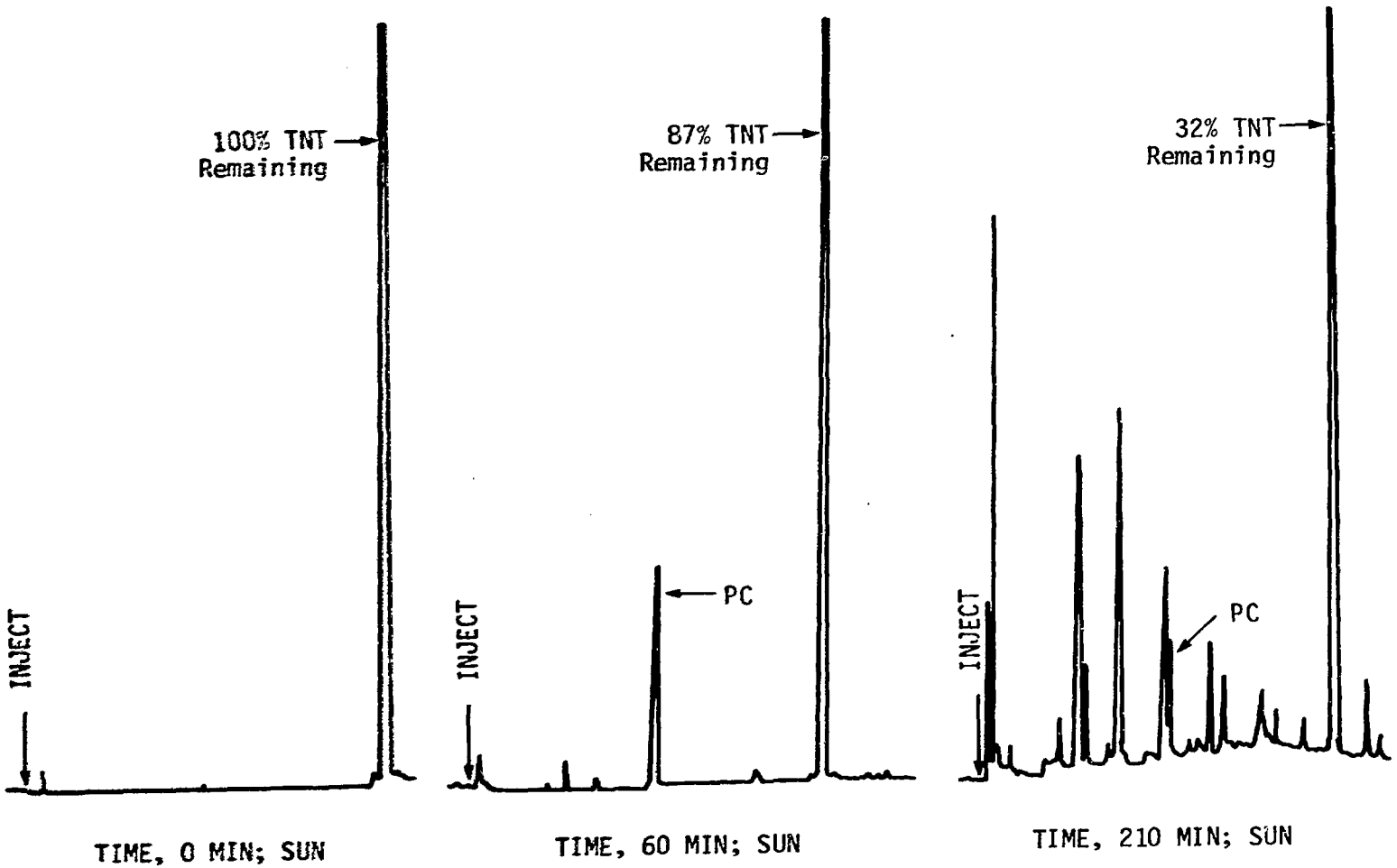


FIGURE 7 TNT PHOTOLYSIS IN SUNLIGHT. HPLC: 0→50% ACETONITRILE:WATER 30-MIN LINEAR PROGRAM FLOW, 2 ML MIN^{-1}, 254 NM

These rate constants fit an Arrhenius expression, $\log k_1 = 12.6 - 24/\phi$, where $\phi = 0.00457\ T$. The entropy term, 12.6, is consistent with a cyclic unimolecular process, and the activation energy, 24 kcal $mole^{-1}$, suggests cleavage of moderately strong bonds. These results are not consistent with a simple proton-transfer reaction, as is the case of the generation of the aci-form of TNT, but indicate more complex molecular interactions.

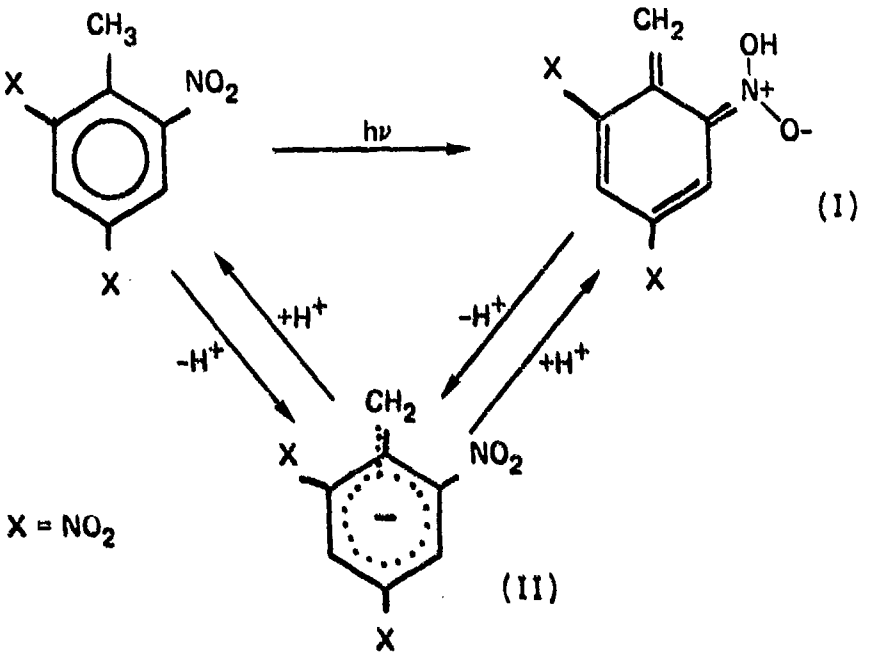
Further characterizations show that PC is more stable in solution at pH 2 than at higher pH values. Although PC reverts to TNT at pH 1 in the dark, rapidly eluting acidic products are the major components according to reverse-phase HPLC analysis. At high pHs (> 12) PC is very unstable; an aqueous PC solution turns purple when raised to pH 12; subsequent neutralization and HPLC analysis of this solution showed only TNT.

When 1 ppm TNT was photolyzed in natural water, no PC was detected in the HPLC analysis; when PC was added to a natural water it was less stable than it was in distilled water--with a half-life of 5 minutes in Searsville Pond water and room temperature and one of 19 hours in distilled water. At higher TNT concentrations (> 60 ppm) some PC did form in natural waters. These results suggest that humic materials may suppress PC formation, or that PC if it does form, may either revert to TNT or photolyze rapidly to other products.

The effect of PC on the photolysis rate of TNT was also studied. A solution of 0.25 ppm PC and 1.0 ppm TNT in 30% acetonitrile-70% water was prepared along with a 1-ppm TNT solution in 30% acetonitrile-70% water as a control. Both of these solutions were photolyzed simultaneously at 313 nm, and the TNT in the PC-TNT solution photolyzed twice as fast as that in the control. This increase in rate suggests that PC may be partially responsible for the rate acceleration observed during TNT photolysis; however, because more PC was present in this experiment than is formed in the usual TNT photolysis, and because in natural water the TNT rate acceleration is about 10 times greater than that in

distilled water, the PC must not be the only product catalyzing TNT photolysis. Burlinson et al. (1979) proposed the following scheme for the partial photolysis of TNT. This mechanism does not explain the loss of TNT, only the photocatalyzed hydrogen-deuterium exchange in D_2O.

MECHANISM OF TNT REACTION



Scheme 1

Fyfe et al. (1976) measured the UV and nuclear magnetic resonance (NMR) spectrum for the TNT anion II and found the visible spectrum to have a λ_{max} at about 500 nm and the NMR spectrum to have peaks at δ 5.4 and 8.1. By comparison, the NMR peaks observed for PC are δ 5.4 (d), 6.4 (d), 8.4 (s), and 8.7 (s) in ratios of approximately 2:2:1:3 or 1:1:1:2. The UV-visible spectrum of PC has a broad band centered at approximately 500 nm. The lower wavelength absorption of PC is uncertain because of the TNT contaminating all PC samples, but it does appear to be a broad band without any marked maximum.

Attempts were made to obtain the mass spectrum of PC using chemical ionization (CI), field ionization (FI), and electron-impact (EI) mass spectrometers. The major CI and FI peaks obtained were at 391, 278, 227, 210, and 211 AMU. The major EI peak obtained was at 210 AMU. In all mass spectrometry (MS) measurements, a red residue was left on the probe after each analysis, indicating that PC is not very volatile or condenses to higher-molecular-weight products in the spectrometers. The information these mass spectral measurements gives on the structure of PC, therefore, is inconclusive.

Thus, the structure of PC is still unknown. Although the water solubility, NMR spectrum, and possibly low volatility (indicated by the mass spectrometry experiment) suggest a TNT anion-type compound (possibly salt or charge-transfer-complex structures), the structures I and II in Scheme I seem unlikely. Burlinson et al. (1979) noted that the aciquinoid structure, I, should have a pKa of about 1; if it does, then the anion II should be formed rapidly in the near-neutral pH solutions used in our studies. However, the pKa of TNT is expected to be greater than about 11, so it is also unlikely that II could be isolated from neutral solutions such as those used in our studies. More laboratory work is needed to determine the structure of PC and its role in the overall phototransformation of TNT.

4. Biotransformation

a. Screening Test

TNT (10 ppm), added with DMSO to Waconda Bay water obtained in July (bottle 1) remained unchanged for 20 days (lag period), then decreased in concentration to about 3 ppm after 64 days of incubation. The TNT disappearance followed first-order kinetics with a half-life of 25 days estimated from the curve in Figure 8. The TNT in Waconda Bay water collected in August (bottle 2) had a lag phase of 13 days, then decreased to about 1 ppm at day 68. A half-life of 19 days was estimated from the curve in Figure 9. The bacterial counts of the water in bottle 1 were 1.3×10^6, 1.4×10^6, and 8.2×10^5 cells ml^{-1} at days 43, 54, and 64, respectively, and those of the water in bottle 2 were 1.35×10^6, 9.6×10^5, and 9.0×10^5 cells ml^{-1} on days 27, 37, and 52. One bottle of water collected in July was initiated with TNT and 30 ppm of yeast extract and another 30 ppm of yeast extract was added on day 26 (bottle 3, Figure 8). TNT in this bottle degraded without a lag period and decreased to 1.5 ppm at 43 days. With the August water and 1% (dry weight) sediment (bottle 4, Figure 9), TNT also biotransformed without a lag phase. The half-life in this bottle was 18 days. The bacterial counts in this water were 8.7×10^6 and 8.3×10^6 cells ml^{-1} on days 52 and 71, respectively.

In an anaerobic experiment with water collected in July (sample under N_2 gas atmosphere), the rate and lag time of TNT transformation were about the same as those in the aerated bottles. Therefore, TNT transformation is not accelerated under microaerobic conditions.

Because TNT appeared to be biotransformed, the microorganisms in these experiment bottles were transferred to TNT media to develop enriched cultures for kinetic studies. When TNT was about 50% transformed in bottle 1, aliquots of the water were inoculated into shaker flasks containing 10 ppm TNT (with DMSO) and basal-salts medium. An increase in turbidity was observed after a week of incubation, and

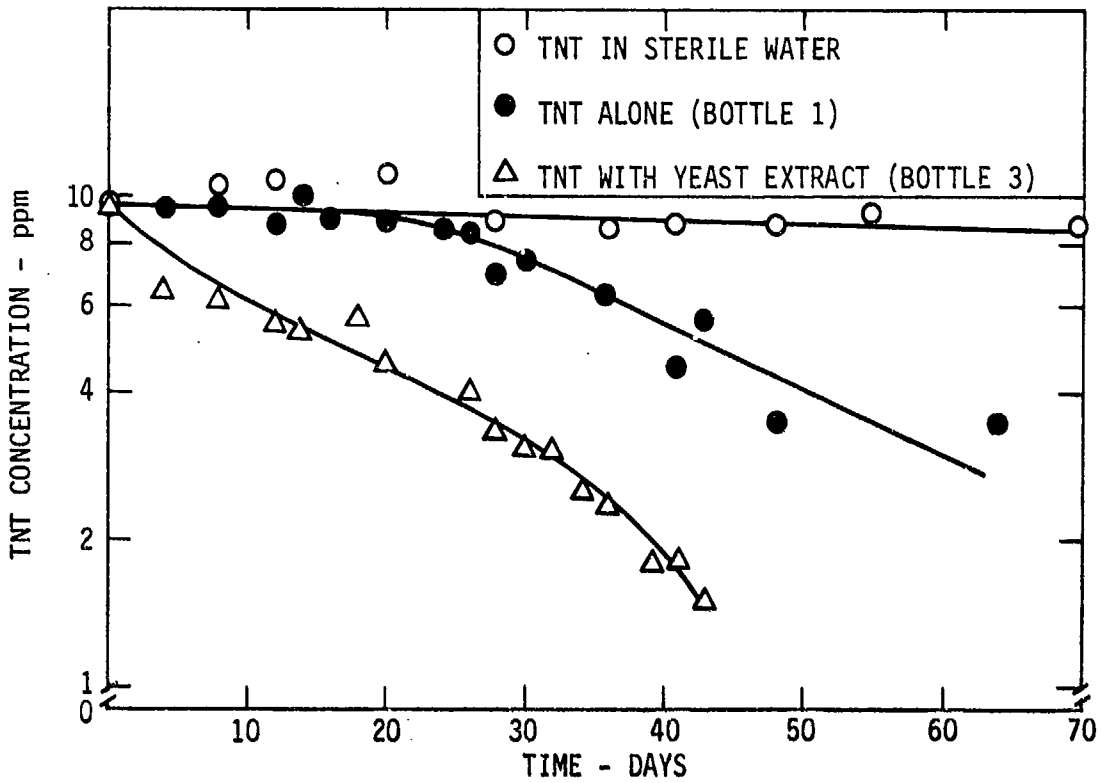


FIGURE 8 BIOTRANSFORMATION OF TNT IN WACONDA BAY WATER COLLECTED IN JULY (TNT ADDED WITH DMSO)

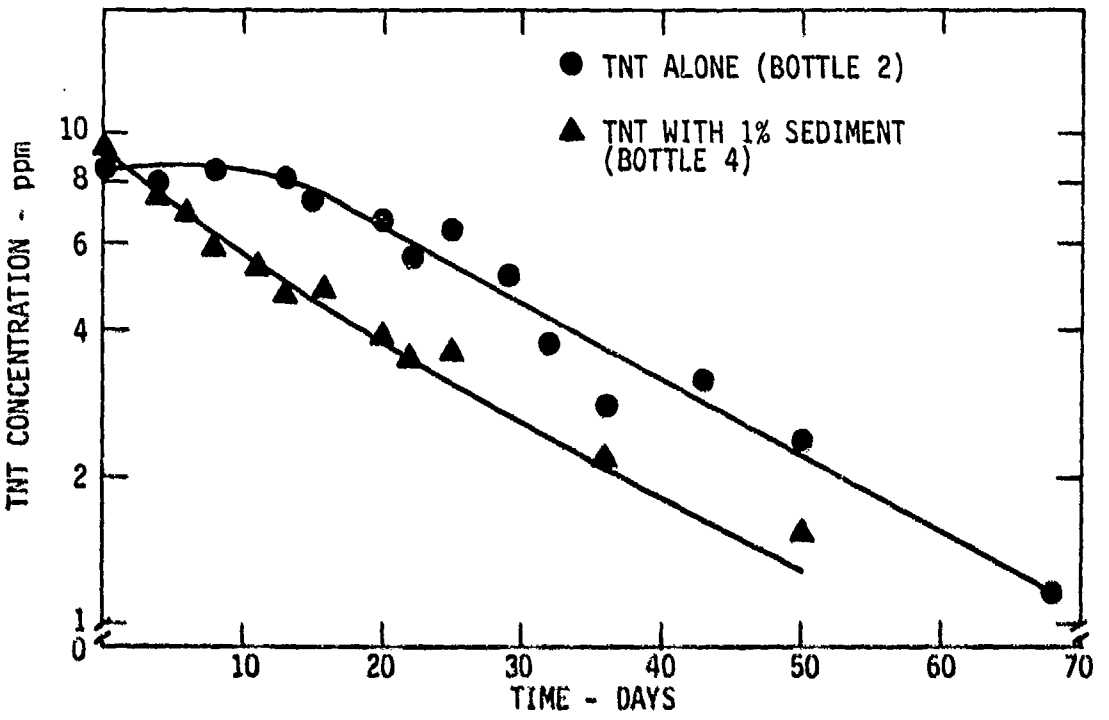


FIGURE 9 BIOTRANSFORMATION OF TNT IN WACONDA BAY WATER COLLECTED IN AUGUST (TNT ADDED WITH DMSO)

the TNT was found to be transformed. In these transfer flasks, 10 ppm of TNT was reduced to 5 ppm after several days of lag time and 1 to 2 weeks of incubation.

When the water containing 1% sediment in bottle 4 was also inoculated into flasks with 10 ppm TNT and 1% Waconda Bay sediment in basal-salts medium, the TNT decreased to 5 ppm in 5 days and to 3 ppm in 7 days. When organisms from the water with yeast extract (bottle 3) were transferred to a flask with 60 ppm yeast extract containing TNT and basal-salts medium, the TNT decreased from 10 ppm to 5 ppm in 7 days.

These results indicate that TNT can be transformed slowly without additional organic nutrients, although sediment or yeast extract may provide extra organic nutrients for faster cell growth and TNT transformation with shorter lag phases. However, in each case, TNT was first dissolved in DMSO and then added to water or media because DMSO is a convenient solvent and because it is generally resistant to biotransformation and is nontoxic at the level used. From our past experience, we judge that the use of DMSO did not interfere with biotransformation.

Because the TNT (DMSO)-basal-salts medium solution was more cloudy than solutions of microbes grown on 10 ppm of TNT (without DMSO), slowly growing TNT-transforming microorganisms could have been utilizing DMSO. To investigate this possibility, a TNT-transforming microorganism mixture was inoculated into DMSO in basal-salts medium (250 µl-liter) without TNT. Cell growth was assessed after a week of incubation by visual inspection of cloudiness and microscopic examination and indicated that DMSO is being utilized.

To eliminate the possible effect of DMSO, a microorganism mixture was inoculated into basal-salts medium containing 10 ppm TNT but without DMSO. The TNT concentration in this flask decreased to 7 ppm in 2 weeks, probably because nutrients or active enzymes were carried over from the inoculum. In the medium with TNT and DMSO, TNT was reduced to 3 ppm in 2 weeks. In another experiment, microorganisms in the

TNT-screening test bottle were inoculated into the flasks containing TNT-basal-salts medium (without DMSO), TNT-basal-salts medium plus yeast extract (1 g-liter), or Difco nutrient broth (1 g-liter). The TNT concentration of 12 ppm was unchanged in the uninoculated control flask and in the flask with TNT alone for 30 days. However, in the medium containing yeast extract and in that with nutrient broth, the TNT concentrations dropped to 1.7 ppm and 1.0 ppm, respectively, by day 9.

It is apparent that TNT was biotransformed rapidly with extra organic nutrient, including DMSO, which seems to be slowly metabolized by the acclimated microorganisms. The lag period may be the time required for the organisms to acclimate to DMSO. The organisms either could not transform or were very slow in transforming, TNT when no other organic compounds were present to provide nutrients.

To study further the effect of DMSO on TNT biotransformation in natural water, local eutrophic pond water was collected and 10 ppm of TNT was added with and without DMSO (250 $\mu g\ liter^{-1}$). The TNT in the bottle with DMSO began to decrease after a 29-day lag phase and yielded a 16-day half-life; this solution reached 2 ppm TNT in 67 days. TNT without DMSO showed a lag phase of 40 days and reached 5 ppm after over 110 days of incubation (Figure 10). The half-life estimated from the slope of the first-order plot was 80 days in this case which indicates that DMSO did affect TNT transformation in natural water in the screening test bottle. In these examples, the bacterial counts were 1-1.5 $\times$ 10^6 cells ml^{-1} at lag phase in both water samples, increasing to 3 $\times$ 10^6 cells ml^{-1} in the water with DMSO, but remaining at 1.4 $\times$ 10^6 cells ml^{-1} in the water without DMSO during the TNT transformation period.

To determine the effect of amount of organic nutrient on the rate of TNT degradation, 10 ppm TNT in basal-salts medium containing 10, 100, or 1000 ppm yeast extract was inoculated with Waconda Bay TNT-transforming microbes in shaker flasks (Figure 11). In the presence of 1000 ppm yeast extract, 50% of the TNT was transformed in 1 day and less that 10% remained after 4 days. With 100 ppm yeast extract, the

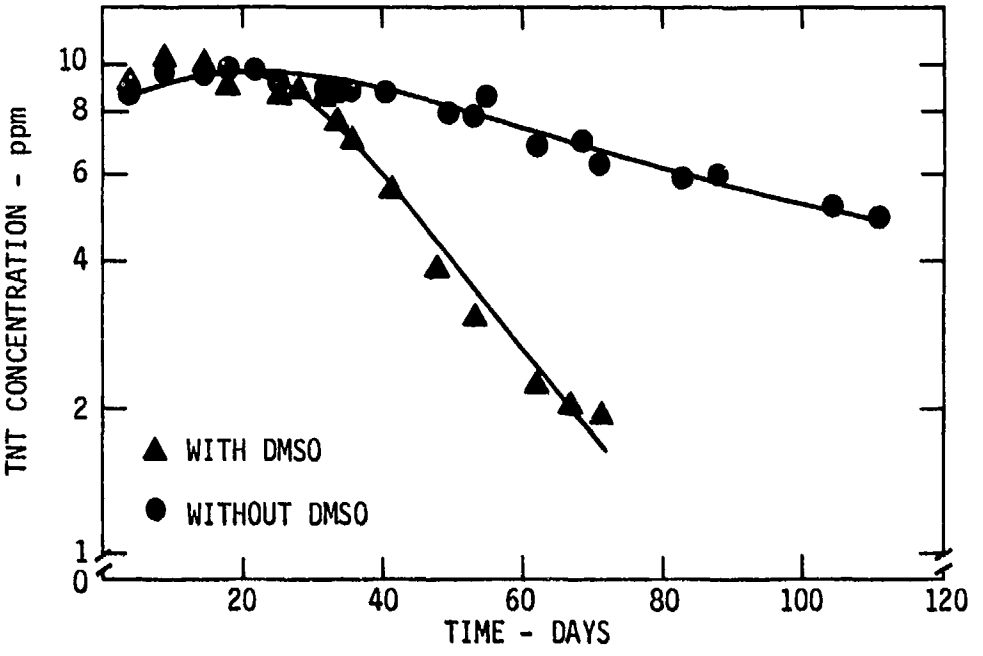


FIGURE 10 BIOTRANSFORMATION OF TNT IN EUTROPHIC POND WATER

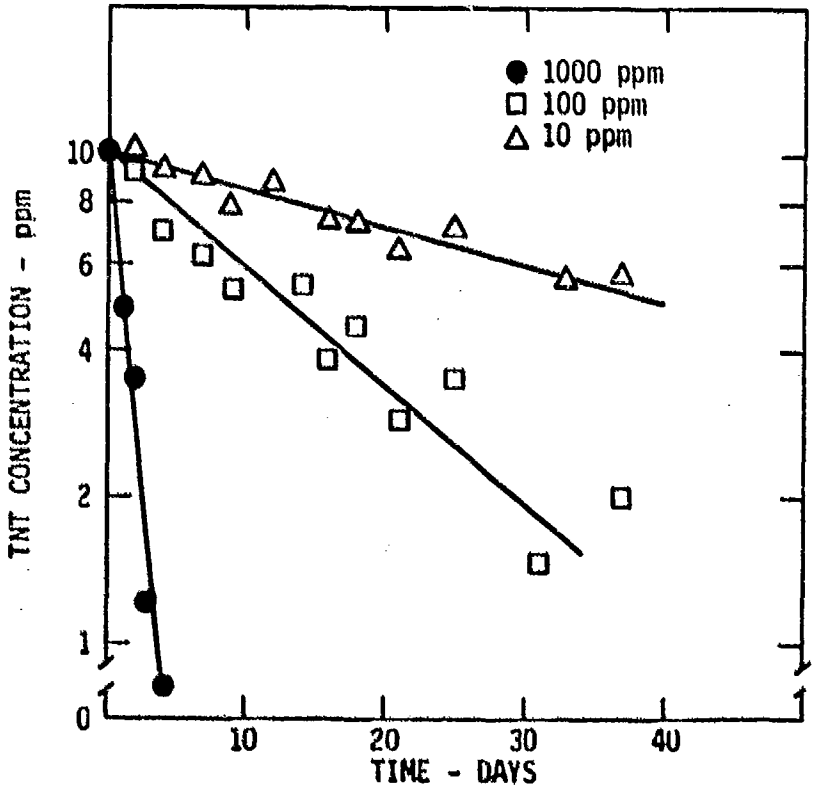


FIGURE 11 TNT TRANSFORMATION IN DIFFERENT CONCENTRATIONS OF YEAST EXTRACT

half-life estimated from the slope of the first-order plot was 13 days, and with 10 ppm yeast extract, the half-life was estimated to be 40 days (Figure 11). The amounts of metabolized organic nutrients, which provide for bacterial growth, play important roles in the TNT transformation rate. Substantial amounts of organic nutrients are required for high transformation rates of TNT to be observed.

During the TNT biosorption study, it was observed that TNT was transformed by a mixture of four ATCC cultures used for the study. In this case, the organisms were grown overnight in Trypticase Soy broth media (without TNT), centrifuged, washed, and resuspended in phosphate buffer. TNT metabolites were detected in TLC and HPLC chromatograms of the supernatant when TNT was exposed to viable cells, but not in that of TNT exposed to heat-killed cells. Waconda Bay microorganisms were prepared under the same conditions, and 10 ppm TNT was added to cell suspensions of 0.6, 0.14, and 0.03 mg ml^{-1} (dry weight). In the cell suspension of 0.6 mg ml^{-1}, 10 ppm TNT in the supernatant dropped to 0.3 ppm in 1 hour; in the cell suspension of 0.14 mg ml^{-1}, the TNT concentration was 3.8 ppm in 1 hour and 0.8 ppm in 4 hours; in the cell suspension of 0.03 mg ml^{-1}, the TNT concentration was 7.8 ppm after 1 hour and 6.9 ppm after 4 hours. These results and the results with biosorption test bacteria showed that many microorganisms, when grown in organic nutrient-rich media, have TNT-transformation enzymes (e.g., organic nitro reductase) that react with TNT, and that acclimation to TNT is not necessary.

Our experiments with 2,4-DNT showed that it was ultimately transformed by microorganisms (described in another section of this report). To investigate the possible special cometabolic transformation of TNT with 2,4-DNT as a cosubstrate, TNT and yeast-extract-transforming microorganisms, or 2,4-DNT-transforming microorganisms were inoculated into basal salts medium containing 10 ppm TNT and 100 ppm DNT. There was no enhanced consumption of TNT observed in the flasks of either inoculum. The TNT concentration was 7.5 ppm with DNT-transforming organisms and 9.5 ppm with TNT-transforming organisms after 18 days of

incubation. The growth was poor with the latter organism, indicating that the organisms were probably not well adapted with 2,4-DNT as the substrate.

b. Transformation of Ring-Labeled ^{14}C-TNT

A study was conducted to determine if the benzene ring in TNT was cleaved during transformation. In earlier experiments, more than 90% of the TNT was biotransformed in 4 days in the medium containing 10 ppm TNT and 1000 ppm yeast extract. In the sealed flask system containing this same medium, substantial amounts of CO_2 were evolved in the KOH trap during the first 3 days of incubation because of the microbial growth on yeast extract, and very little CO_2 was produced after 5 days. However, total $^{14}CO_2$ evolved during 7 days of incubation was less than 0.5% of the added ^{14}C, with 99% of the added activity found in the medium. More than 95% of the TNT was transformed during this time.

After 7 days of incubation, the broth was extracted twice with ethyl acetate (fraction 1), acidified to pH 2, and re-extracted (fraction 2). The distribution of radioactivity in each fraction was 46%, 21%, and 33% in fraction 1, fraction 2, and the aqueous phase, respectively. TLC analysis of the solvent extractions showed the presence of monoamino-, diamino-, and azoxy-derivatives of TNT (see Metabolites below).

This result shows that the nitro group of TNT was reduced by microorganisms, but the ring was not cleaved and TNT will not be mineralized by these organisms.

c. Biotransformation Rate Constant

The results of the screening test showed that TNT transformed very slowly in natural water even with small amounts of extra organic nutrients. No enriched mixed culture that can use TNT as a sole carbon source was obtained from the Waconda Bay water.

During the screening test, microbial concentrations in the waters were fairly constant, and TNT transformations generally followed first-order kinetics. Although organic compounds other than TNT were required

for transforming TNT, a transformation rate constant may still be calculated under these conditions assuming that the organic nutrient is only present to effect cell growth. The rate constants calculated from the results obtained with TNT in natural waters using equations 14 and 15 are shown in Table 14. Average cell counts were used as listed in the table.

From these data we calculated that the second order rate constant, based on total bacterial counts, ranged from 1.8×10^{-10} to 1.4×10^{-9} ml $cell^{-1}$ h^{-1}, which means that the biotransformation of TNT is relatively slow.

Burlinson (1978) conducted river die-away degradation tests of TNT with a Potomac River water. In darkness 90% of the original 20 ppm TNT disappeared in 30 days. His data indicate that TNT disappearance followed first-order kinetics, and that the half-life was 8 days, with a pseudo-first-order rate constant of k_b^1 of 3.6×10^{-3} h^{-1}. The total bacterial count was about 1×10^6. Therefore, the second-order rate constant was 3.6×10^{-9} ml $cell^{-1}$ h^{-1}, a value consistent with our results.

In an experiment with high cell concentrations, the TNT yeast-extract degrading organisms were grown in Trypticase Soy broth medium overnight, centrifuged, washed, and resuspended in basal-salts medium. TNT was added to 2 different levels of cell suspensions, and TNT was analyzed periodically. The plate counts of 2 suspensions were 9.0×10^8 and 1.7×10^9 cells ml^{-1} (Figure 12). The half-lives of TNT in these suspensions, estimated from the slopes, were 5.6 hours and 4.0 hours, respectively. Thus, the pseudo-first order rate constants, k_b^1, were 0.12 $hour^{-1}$ and 0.17 $hour^{-1}$, respectively, and the second-order rate constants were 1.4×10^{-10} and 1.0×10^{-10} ml $cell^{-1}$ h^{-1}, respectively. These rate constants are in good agreement with those found for natural water.

d. Metabolites

As indicated by the experiment with ^{14}C-labeled TNT, TNT undergoes only limited transformation; most of the products retain the benzene ring. TLC of the ethyl acetate extract of TNT solutions exposed

Table 14

BIOTRANSFORMATION RATE FOR TNT IN THE SCREENING TEST WATERS

Water	Substrate	Lag Time (Days)	Cells $\times 10^{-6}$ ml^{-1}	$t_{1/2}$ (Days)	$k_{b_1} \times 10^{3}$ h^{-1}	$k_{b_2} \times 10^{9}$ ($ml\ cell^{-1}h^{-1}$)
Waconda Bay water 1	TNT (10 ppm) with DMSO	20	1.2	25	1.3	1.1
Waconda Bay water 2	TNT (10 ppm) with DMSO	13	1.1	19	1.5	1.4
Waconda Bay water with 1% sediment	TNT (10 ppm) with DMSO	--	8.5	18	1.6	0.18
Searsville Pond water	TNT (10 ppm) with DMSO	33	3.0	16	1.8	0.60
Searsville Pond water	TNT (10 ppm)	40	1.4	8	3.6	0.26

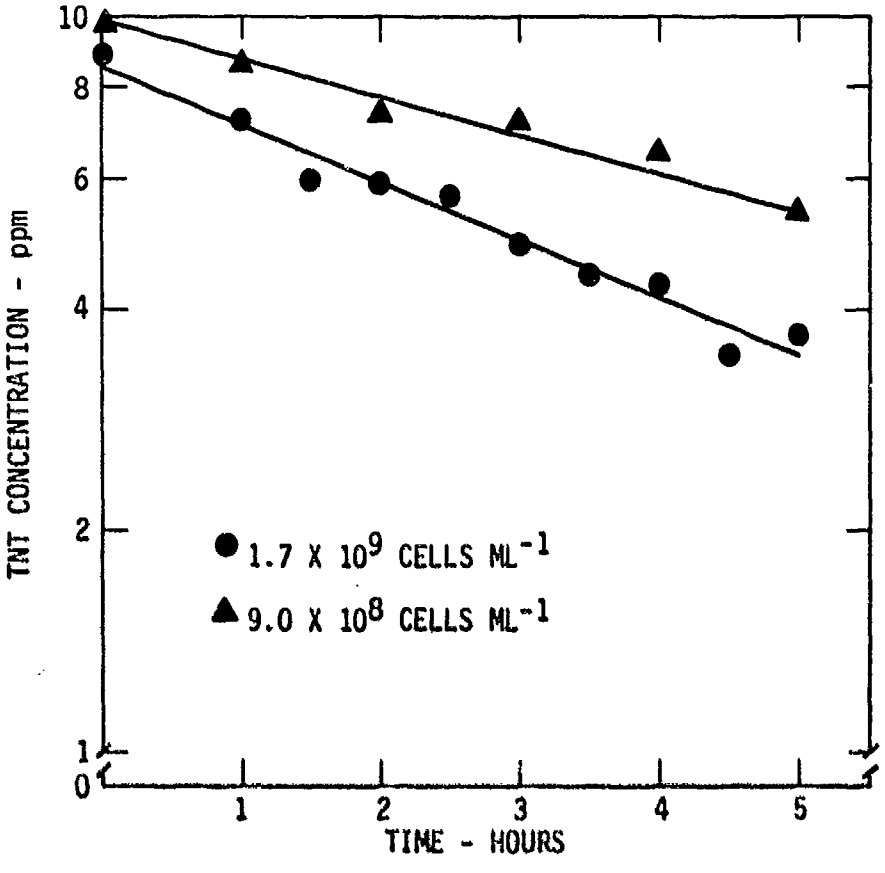
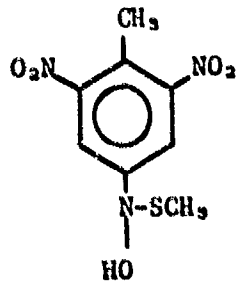


FIGURE 12 TNT BIOTRANSFORMATION BY WASHED CELLS GROWN IN TRYPTICASE SOY BROTH

to Waconda Bay organisms showed the presence of many metabolites (Figure 13). By comparative TLC with known standards, 5 components were identified as 2-amino-4,6-dinitrotoluene (2A), 4-amino-2,6-dinitrotoluene (4A), 2,4-diamino-6-nitrotoluene (2,4-DA), 2,6-diamino-4-nitrotoluene (2,6-DA), and 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4-AZO). The identities of 2A and 4A were confirmed by gas chromatography-mass spectrometry (GC-MS) and the identity of 4-AZO was confirmed by probe MS.

To try to identify some of the more reactive intermediates, the TLC profiles of the TNT biotransformation solutions were compared on days 2 and 8. An intermediate present in a large quantity on day 2 but less on day 8 (R_f 0.23 to R_f 0.35) was scraped from the plate and evaluated by high-resolution MS. A molecular weight of 254.0271 was obtained, which corresponds to a molecular formula of $C_8H_9N_3O_5S$. This formula can be represented as 1 or 2. Thus it appears that a sulfhydryl group is playing a role in the reduction of the nitro group. Saz and Slic (1953) reported that the reduction of nitroaromatic compounds in



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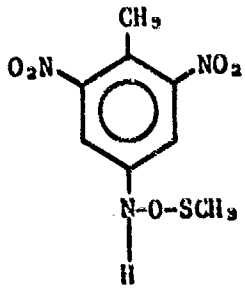

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cell-free extracts of E. coli depends on cysteine, which may indicate that cysteine is the source of sulfur in compounds 1 and 2. Thiomethyl group of methionine or its decomposition product, methanethiol, may be other sources of this group.

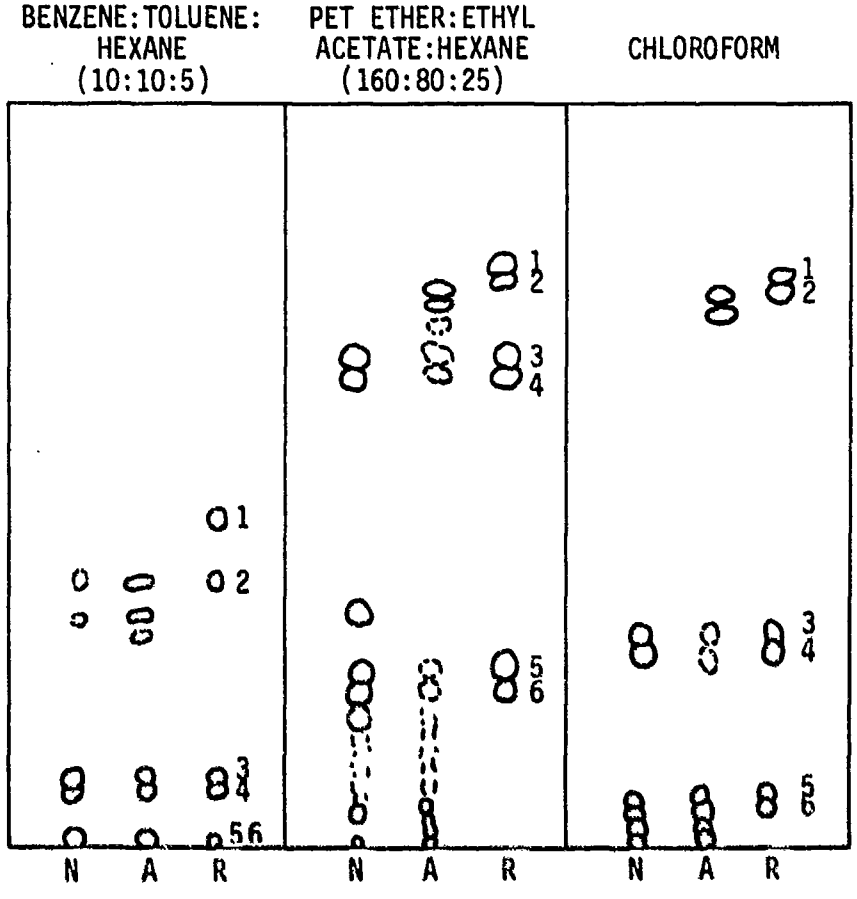


FIGURE 13 THIN-LAYER CHROMATOGRAM OF TNT METABOLITES

Sample: N, Neutral extract
A, Acidic extract
R, Reference chemicals

Compounds: 1, TNT
2, 4-AZO
3, 4A
4, 2A
5, 2,6-DA
6, 2,4-DA

To study the effect of cysteine or methione on TNT transformation, TNT-biodegradation microorganisms were grown in the basal-salts medium with 50 ppm TNT and 5000 ppm yeast extract for 2 days, centrifuged, washed, resuspended, and used for the TNT transformation test. The flasks with cells in basal-salts medium and 10 ppm TNT were enriched with 100 or 500 ppm of cysteine, methionine, or yeast extract. The results (Figure 14) show that the TNT concentrations in the TNT control preparations and in cysteine- or methionine-enriched media were not significantly different, but that in the medium with 100 and 500 ppm yeast extract, TNT decreased significantly. Either cysteine and methionine are not directly involved in TNT nitro group reduction, or the washed cells contained enough sulfur-containing amino acids for TNT transformation. The enhanced TNT biotransformation in the presence of yeast extract implies that some factors or cofactors present contribute to accelerated enzyme activity in the transformation.

The ethyl acetate extract of the broth was also investigated by HPLC (Fig. 15). This technique confirmed the presence of 4-AZO, 2A, 4A, 2,4-DA, and 2,6-DA. There are small amounts of 1,3,5-trinitrobenzene and 3,5-dinitroaniline, but whether the production of these compounds is due to a biological process, a photochemical process, or a combination of both processes is not clear.

To further investigate metabolites, several of the major components shown in Figure 15 were collected from the HPLC column and probed by MS. Peak 3, which was tentatively identified as 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4-AZO) by comparing retention time to that of an authentic standard (obtained from N. Burlinson, NSWC) was confirmed by MS analysis (M/e = 406). Peak 9 was found to be isomeric with Peak 10 and, based on the mass fragmentation pattern, was tentatively identified as 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2-AZO). Peak 8, which consisted of two components, also showed a molecular ion at M/e = 406 and was tentatively identified as compound 3 and/or compound 4 (2',4-AZO or 2,4'-AZO). The mass spectra of these components appear in Figures 16, 17, and 18.

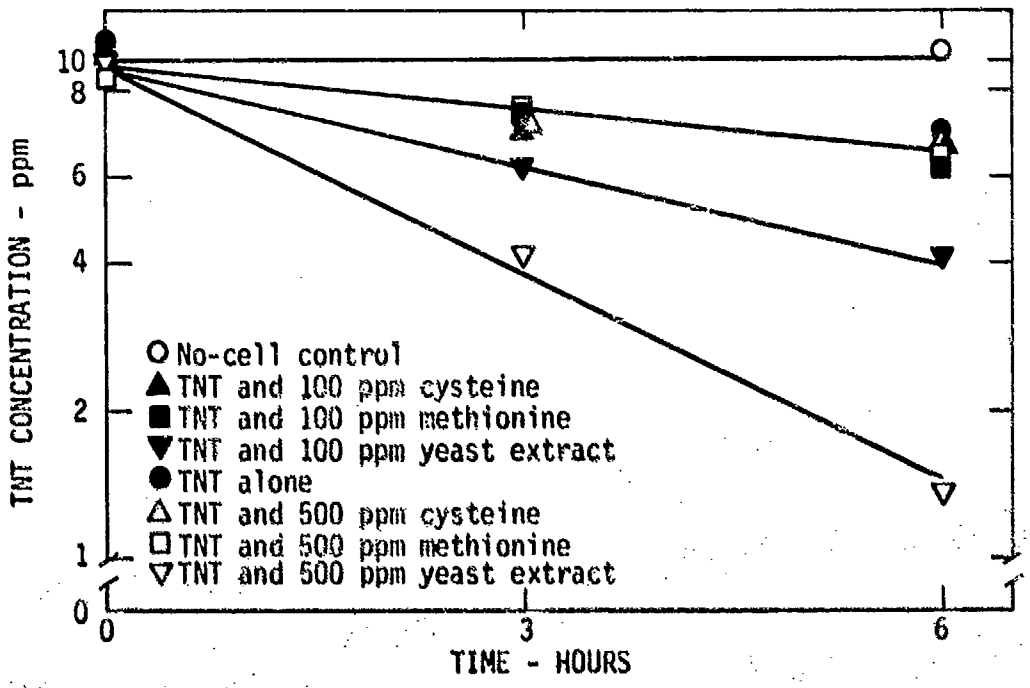


FIGURE 14 TNT BIOTRANSFORMATION WITH WASHED CELLS AND CYSTEINE, METHIONINE, OR YEAST EXTRACT

T-50 extract

100% H_2O → 70%

CH_3OH in 15 min

Linear gradient

2 ml min^{-1}

UV 254 nm

Neutral extract

Possible constituents:

(1) 2,6-Diamino-4-nitrotoluene

(2) 2,4-Diamino-6-nitrotoluene

(3) 1,3,5-TNB

(4) 3,5-Dinitroaniline

(5) 2-Amino-2,6-dinitrotoluene

(6) 4-Amino-2,6-dinitrotoluene

(7) 2,4,6-TNT

(8) 4-Azoxy-2,2',6,6'-tetranitrotoluene

(9) 2,4'-Azoxy-4,4,6,6'-tetranitrotoluene

(10) 2-Azoxy-4,4',6,6'-tetranitrotoluene

Based on a ± 5% retention window

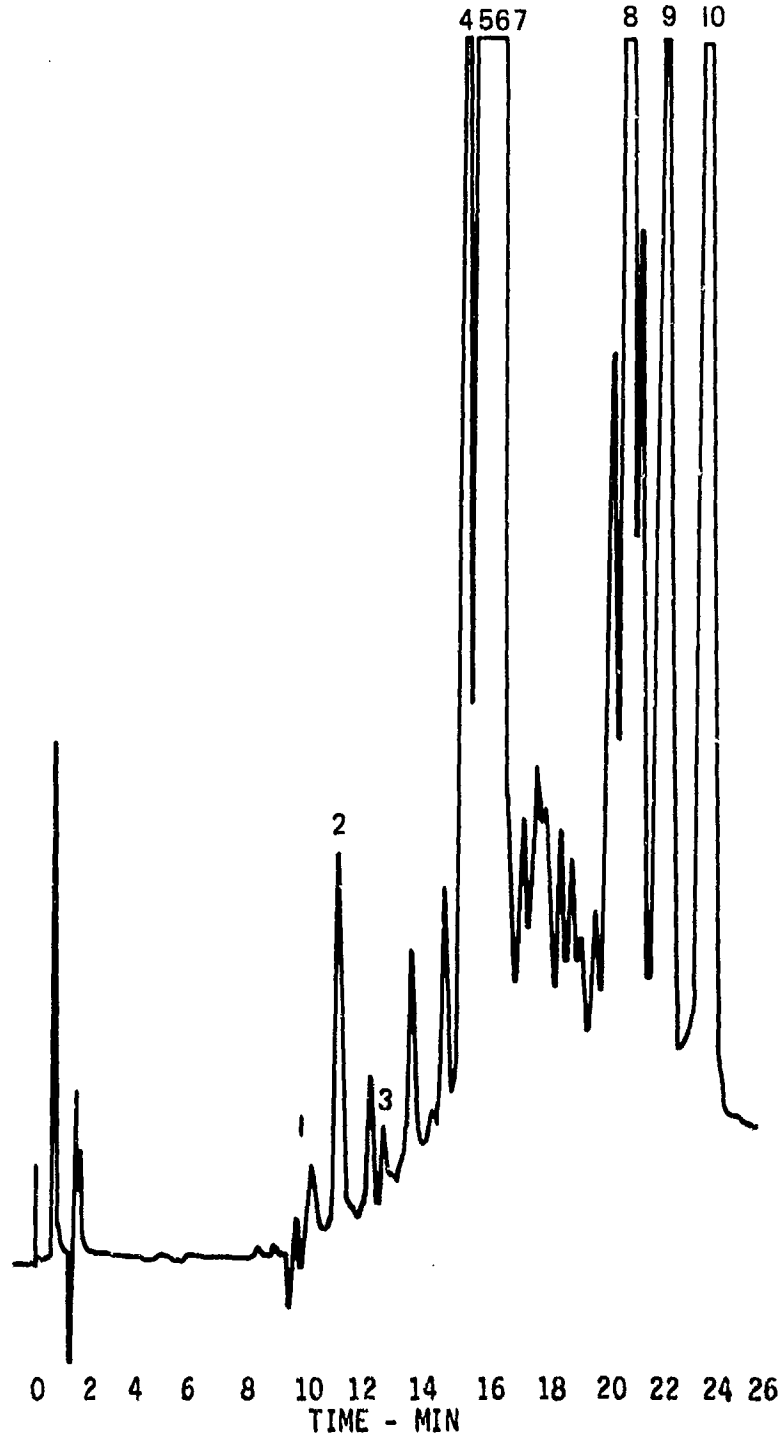


FIGURE 15 HPLC PROFILE OF THE ETHYL ACETATE EXTRACT OF BROTH FROM TNT BIODEGRADATION EXPERIMENTS WITH WASHED CELLS

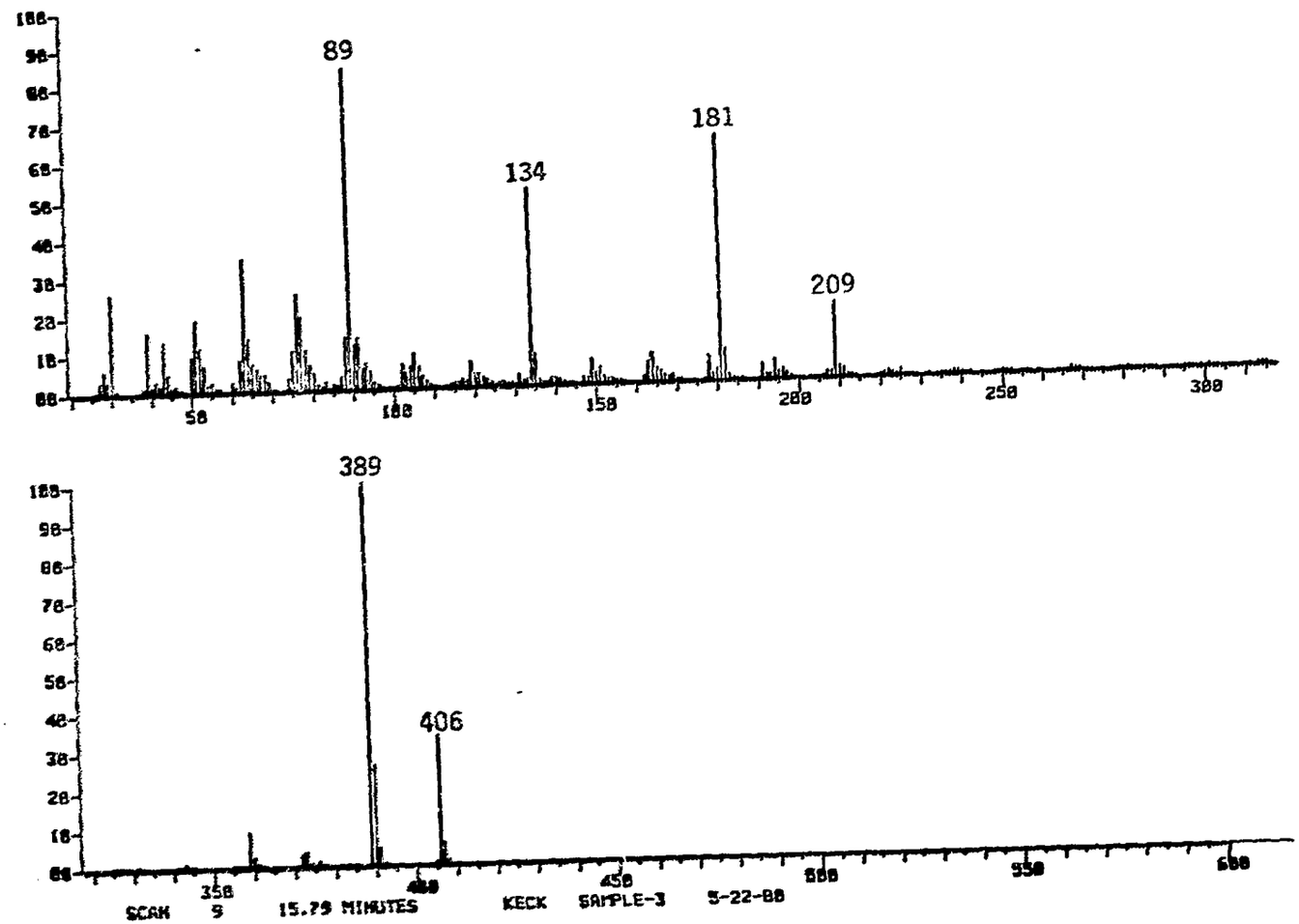


FIGURE 16 MASS SPECTRUM OF 4-AZOXY-2,2',6,6'-TETRANITROTOLUENE

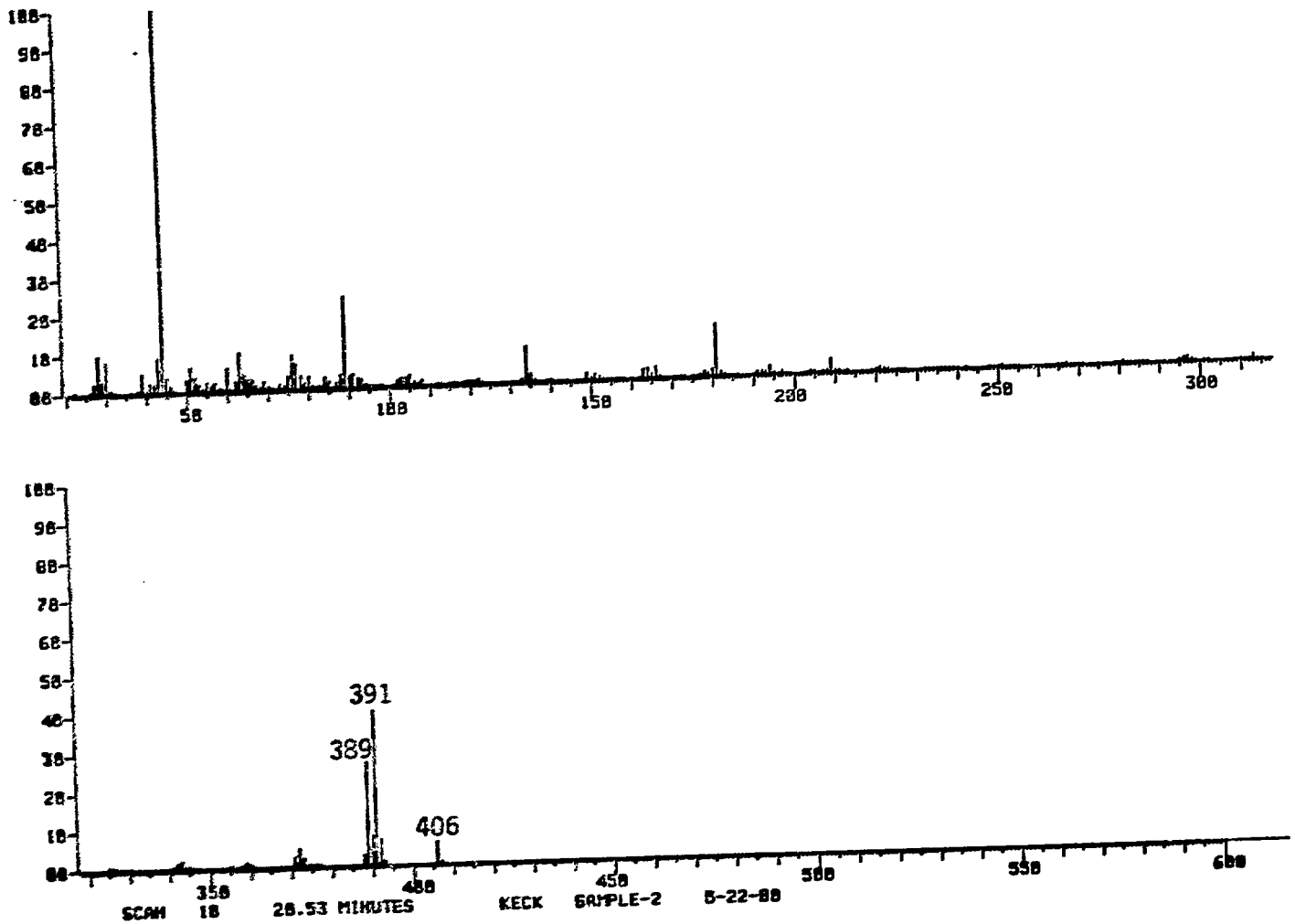


FIGURE 17 MASS SPECTRUM OF TNT METABOLITE TENTATIVELY IDENTIFIED AS 2,4'-Azoxy-2,2',4,6'-tetranitrotoluene

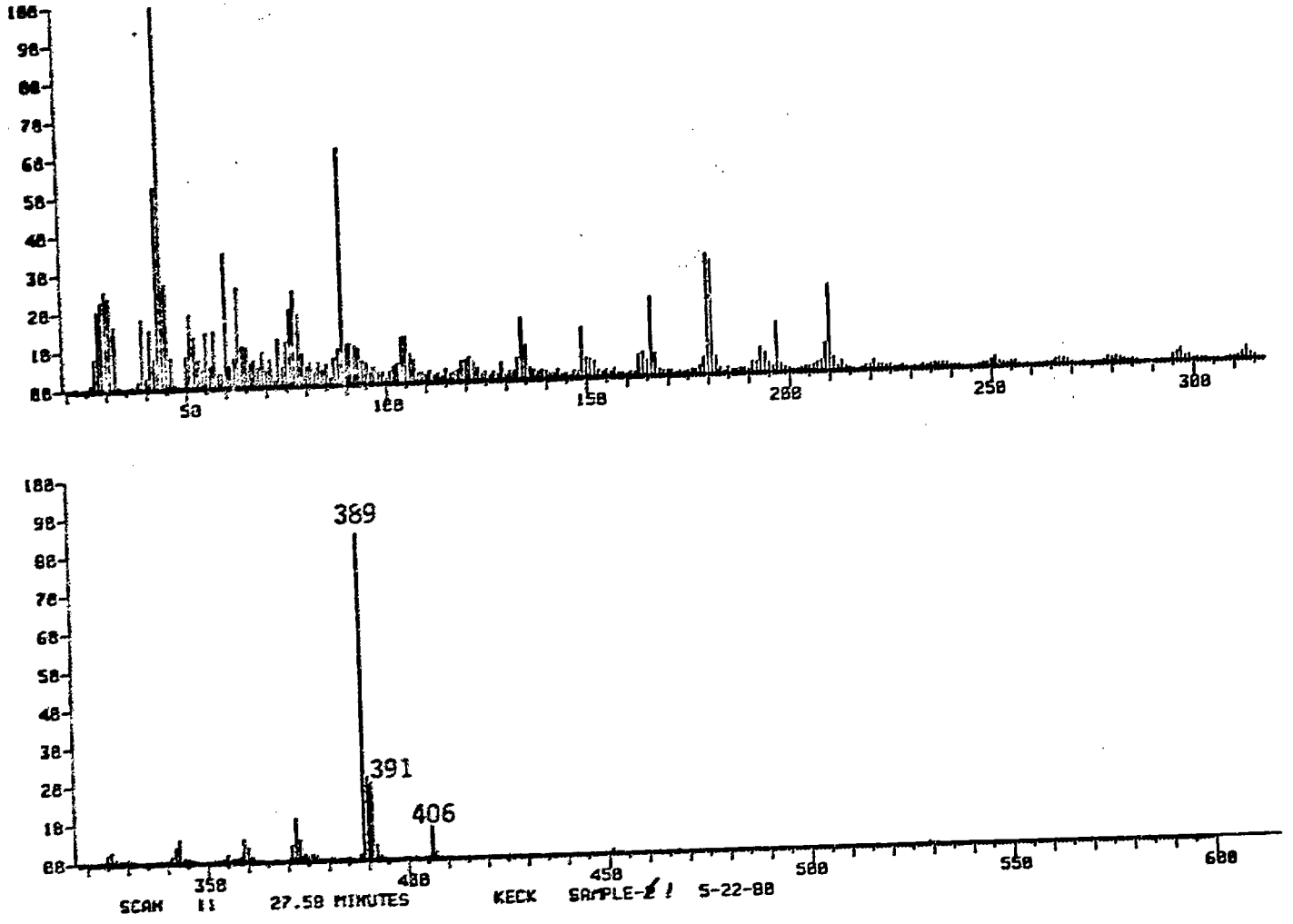
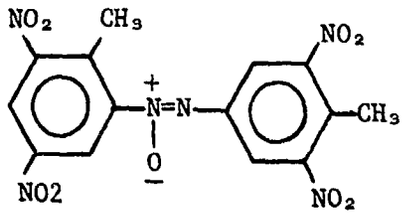


FIGURE 18 MASS SPECTRUM OF TNT METABOLITE TENTATIVELY IDENTIFIED AS 2-Azoxy-4,4',6,6'-tetranitrotoluene



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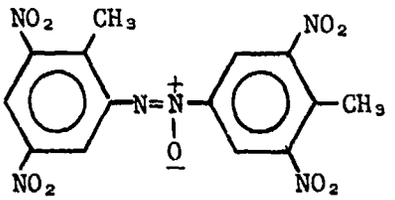

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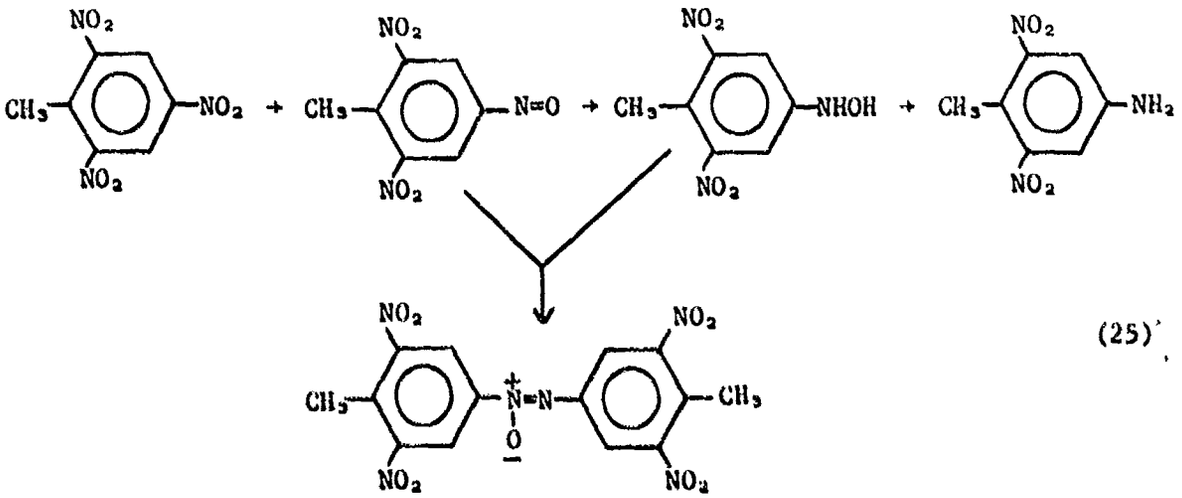
It appears that the formation of these azoxy compounds may result from the condensation of a hydroxylamino compound and a C-nitroso compound. Both of these intermediates arise in the reduction pathway from the nitro group to the amino group.



(25)

Since 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene are formed in nearly equal amounts, there is no preference for reduction to occur at the 2- or 4- position in TNT. This may also result in similar distributions of mixed nitroazoxytoluenes.

To ensure that this condensation was not a result of the extraction and concentration procedures, supernatants from the biodegradation flasks were evaluated by HPLC using direct aqueous injection. Profiles comparable to those of the concentrated extracts were obtained. Therefore, the formation of the azoxy compounds is probably an intracellular process.

e. Discussion

The results showed that the biotransformation of TNT in natural waters is 1000-fold slower than phototransformation. Many microorganisms possess organic nitro-reductase capable of reducing the nitro group of TNT to the amino group when grown in organic nutrient-rich media, but cannot utilize the carbon of the benzene ring of TNT as a sole carbon and energy source. These organisms appear to utilize TNT in a cometabolic process, with other organic nutrients used as cosubstrates. Fast biotransformation rates, corresponding to half-lives of several hours can only be achieved by more than 10^9 microorganism cells ml^{-1} which will require substantial amounts of organic nutrient, on the order of more than 1 g $liter^{-1}$. This is significantly greater than the microorganism concentrations found in the natural water.

That pure cultures or mixed cultures of microorganisms can use TNT as a sole carbon source was reported by Won et al. (1974), Traxler et al. (1974), and Weitzel et al. (1975). In all cases, the transformation was slow, and the addition of organic nutrients such as glucose, yeast extract, peptone, and fatty acids greatly increased the transformation rate. Traxler et al. (1974) also reported that, with an isolated mutant, nitrite ion was produced from the nitro group of TNT. With ring-labeled ^{14}C-TNT, $^{14}CO_2$ was produced. However, the activity in the $^{14}CO_2$ accounted for only 0.3 to 1.2% of added ^{14}C.

Other workers found that TNT was not biotransformed by their TNT degrading organisms without the addition of organic nutrient (Osmon and Klausmeir, 1972; Carpenter et al., 1978). Carpenter et al. (1978)

used ^{14}C-labeled TNT and reported that $^{14}CO_2$ was not found in the trap (less than 0.5%), and that the radioactive compounds in microflora were not the characteristic constituents of lipid and protein materials (fatty acids and amino acids), but were bound to these compounds as polyamide macromolecules. No evidence indicated that the TNT aromatic nucleus was cleaved. Our result was similar to the findings of Carpenter et al. (1978). The mixed culture obtained from Waconda Bay did not include the organisms that can effectively utilize TNT as a sole carbon source. The major process in transformation is nitro-group reduction in the presence of other organic nutrients; ring cleavage is not detected.

The metabolites we found in the microorganisms with TNT and yeast extract medium are monoamino-, diamino-, and azoxy-derivative of TNT that are similar to what other workers found (Won et al., 1974; Weitzel et al., 1975; McCormick et al., 1976; Parrish, 1977; Carpenter et al., 1978; Hoffsommer et al., 1978). One interesting compound found in our metabolite study had a thio-methyl group. This was an unexpected finding, and indicated that some sulfur compound may be involved in the reduction of the nitro group.

5. Biosorption

TNT was biotransformed by the 4 American Type Culture Collection (ATCC, p. 155) cultures used for biosorption tests. When TNT was mixed with viable cells (0.5×1 mg ml^{-1}), more than 80% of the TNT disappeared from the supernatant, but could not be recovered when the pellets were extracted with methylene chloride or ethyl acetate. When ring-labeled ^{14}C-TNT was added to the TNT and mixed with 0.47 mg ml^{-1} of cell mixture, 91.4% of the radioactivity was found in the supernatant, but HPLC analysis showed only 17% residual TNT. An ethyl acetate extract of the cells showed 3.5% of the total activity, and less than 0.5% of the activity remained in the cells after extraction. The supernatants were extracted with ethyl acetate under neutral and acidic conditions, and the TLC plate showed that 4A, 2A, 4-AZO, and possibly 6-AZO were present. Analysis by GC and MS confirmed

2A and 4A. Clearly TNT was transformed by the biosorption test organisms. When TNT was mixed with heat-killed cells, it was not transformed and no metabolites were found.

When the tests of TNT biotransformation by individual ATCC test bacteria were conducted, the initial TNT concentration of 10 ppm was reduced to 4 to 5 ppm in the supernatant with 0.19 to 0.29 mg ml^{-1} of cells, and ethyl acetate extraction of the cells of all organisms showed no detectable TNT by HPLC analysis. All 4 organisms seem to transform TNT, but however, none of the 4 cultures showed especially high levels of activity for TNT transformation.

Many organisms seemed to be able to transform TNT, and it is difficult to find nontransforming organisms; moreover, the biosorption coefficients of chemicals of viable cells and heat-killed cells were not greatly different (Smith et al., 1978). Therefore heat-killed cells were used in the TNT biosorption study. TNT was added at 10 and 20 ppm with cell concentrations of 0.49 and 0.74 mg ml^{-1}. The results are presented in Table 15. The biosorption coefficient of 93 is very low and will not be an important factor in the environment.

Table 15

TNT BIOSORPTION BY HEAT-KILLED BACTERIA

Initial TNT Concentration (ppm)	Cell Concentration (mg ml^{-1})	Sorption Coefficient (Average ± SD)
10	0.49	100 ± 31
	0.74	96 ± 14
20	0.49	88 ± 14
	0.74	87 ± 18
Overall average		93 ± 18

6. Sediment Sorption

The general procedures used to measure the sorption of TNT on natural sediments were described in Background and Methods, in Appendix B, and by Smith and Bomberger (1980).

a. Screening Isotherm

The value of K_{oc} (132) predicted from equation 24a (Table 2), was used to select the appropriate levels of sediment for the screening isotherm. Flasks containing 1 concentration of TNT and 2 concentrations of sediment were prepared. A water blank, TNT blank, and sediment blank were included. No TNT or analytically interfering compounds were found in either the water or sediment blanks. The TNT blank served as a reference for calculating recoveries from the supernatants and sediments. The initial concentration of TNT was 11 μg ml^{-1} and the sediment concentration was 7 mg ml^{-1} in flasks 2 and 3 and 55 mg ml^{-1} in flasks 4, 6, and 7. The flasks were shaken for 15 hours before samples were taken and analyzed. The results given in Table 16 were obtained.

Table 16

AMOUNT OF TNT AFTER 15 HOURS IN SCREENING ISOTHERM

Flask No.	Supernatant (μg)	Sediment (μg)	Total (μg)	Recovery (%)
2	1086	-	1058	-
3	1086	233	1319	121
4	783	194	977	90
6	456	999	1455	134
7	411	978	1389	128

The value of K_p was calculated using equation (22) to be 29.8 ± 6.5 μg g^{-1} μg^{-1} ml (K_{oc} = 29.8 ÷ 0.033 = 900). Later isotherm measurements revealed losses of TNT at longer equilibration times and that the equilibrium of TNT between solution and sediment was not reached until after approximately 140 hours. Because more TNT was sorbed between 15 and 140 h, the estimates of K_p and K_{oc} from the screening isotherm are therefore probably low.

b. Rates of Sorption and Desorption

The rates of sorption and desorption of TNT on Holston River sediment were measured in the following way. A working solution of TNT and TNT blanks (no sediment) were prepared by diluting a stock water solution of TNT in half. Two other samples were prepared by diluting 50 ml of the working TNT solution with 50 ml of a sediment slurry containing 10 g of wet sediment. The flasks containing the samples were shaken, and aliquots were removed at various times to measure the TNT content in the solution and that sorbed on the sediment. The results are presented in Figures 19 and 20. Equilibrium of TNT between solution and sediment is reached after approximately 140 hours (Figure 20). The percent recovery plot in Figure 19 shows a continual loss of TNT during sorption. Because no loss is seen in the flask containing only TNT, volatilization, photolysis, and hydrolysis can be ruled out as mechanisms for TNT loss.

HPLC analysis of the supernatant revealed 1 TNT transformation product. It was not present in the TNT blank or in the sediment, and it increased with time throughout the isotherm. This observation suggests that biotransformation or reaction with the sediment is at least partly responsible for losses of TNT. Irreversible sorption on the sediment, which has been proposed by Sikka et al. (1980), could be responsible for only a portion of the TNT losses. No meaningful data were obtained from the desorption isotherm because the concentration of TNT remaining on the sediment was too low to be accurately measured.

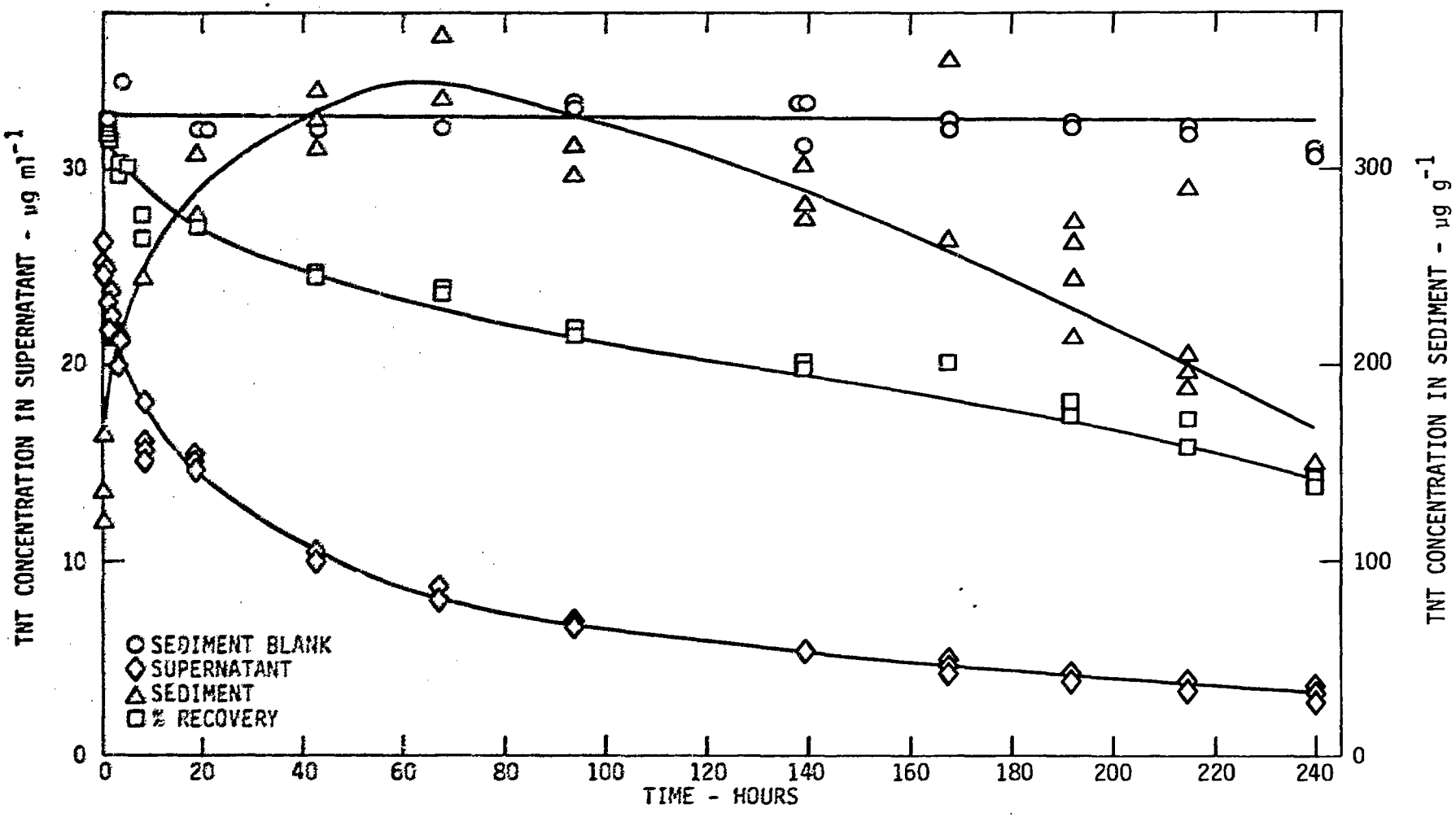


FIGURE 19 RATE OF SORPTION OF TNT TO SEDIMENT, RUN 1

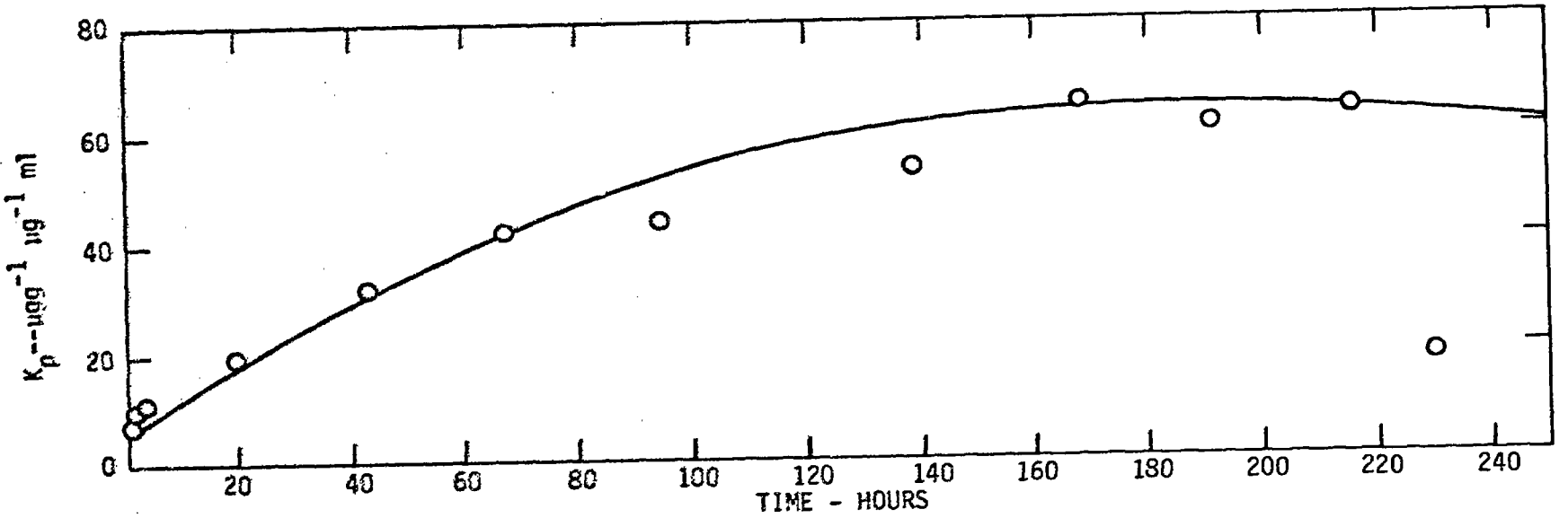


FIGURE 20 K_p VERSUS TIME FOR THE SORPTION OF TNT FROM SEDIMENT, RUN 1

A second sorption-desorption isotherm was measured (run 2). This time $H_{g}Cl_2$ was added in an attempt to retard losses of TNT by biotransformation. The concentrations of TNT and sediment were identical to those used in the first isotherm, except that $HgCl_2$ was added to the flasks. Results similar to those in Figures 19 and 20 were obtained. Loss of TNT still occurred, but the recovery after 350 hours was approximately 60%, compared to the recovery after 350 hours of approximately 20% in the first isotherm measurement. This evidence supports the theory that major losses of TNT in the sorption study are due to biotransformation.

The flasks for a second desorption isotherm were prepared by resuspending the sediment from the second sorption isotherm in deionized water. $HgCl_2$ was added to suppress biotransformation. The results of this isotherm are shown in Figures 21 and 22. The decrease in concentration of TNT in both the sediment and the supernatant in Figure 21 indicates that losses of TNT occurred throughout the isotherm measurement. The partition coefficient (K_p) reached a constant value of approximately 80-90 $\mu g\ g^{-1}\ \mu g^{-1}$ ml after about 100 hours.

e. ^{14}C-Labeled TNT Sorption Isotherm

The isotherm measurements up to this point had all shown losses of TNT. Volatilization, photolysis, and hydrolysis were eliminated as causes of these losses because the TNT blanks containing no sediment were stable. The losses were clearly related to the presence of the sediment. The rate of loss of TNT was retarded by the addition of $HgCl_2$, suggesting that biodegradation is one source of loss. However, irreversible sorption may also cause losses of TNT. A final sorption isotherm was measured to explain more fully the TNT losses.

TNT that had been ring-labeled with C^{14}, was used for the third isotherm measurement. All equipment, including the TNT stock solution and the sediment, was sterilized at the beginning of the experiment. The glassware and sediment were autoclaved for 60 min at 120°C, and the TNT solution was filtered through a 0.22-μ filter. A TNT blank (no

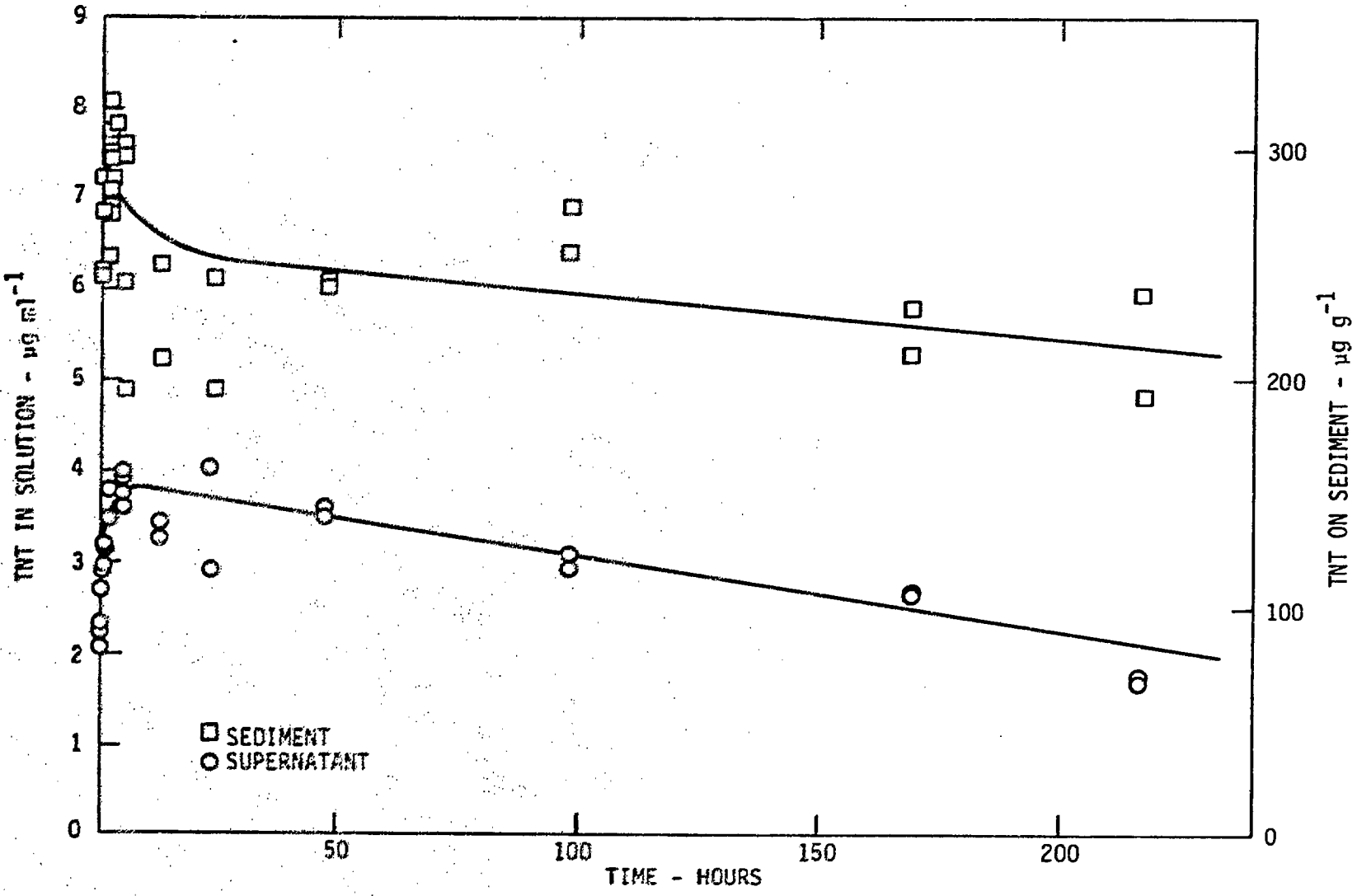


FIGURE 21 RATE OF DESORPTION OF TNT FROM SEDIMENT, RUN 2

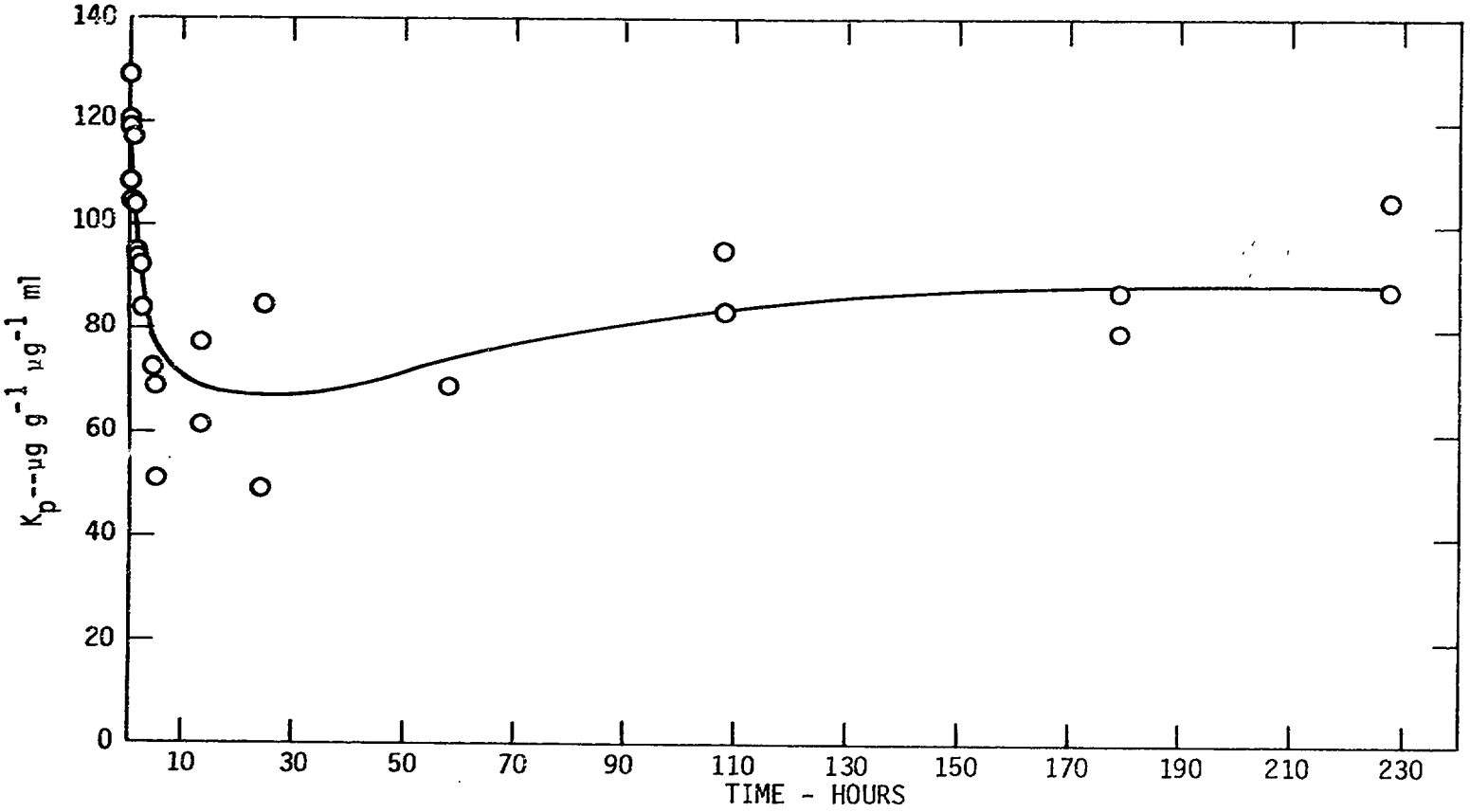


FIGURE 22 TNT PARTITION COEFFICIENT, K_p, AS A FUNCTION OF TIME, RUN 2

sediment), a sediment blank (no TNT), and 2 flasks containing both TNT and sediment were prepared. $HgCl_2$ was added to each flask to inhibit organism growth. Samples were removed at the beginning of the isotherm and after 240 hours and analyzed by HPLC and by counting the radioactivity. The results are summarized in Table 17.

The ^{14}C and HPLC analyses agree very well; the measurement errors are within 3%.

At the beginning of the experiment, essentially all of the TNT was recovered, and none was left on the sediment after extraction with ethyl acetate. After 240 hours, recovery by both ^{14}C and HPLC analysis was below 100%. This could be explained by the error in the analysis, but the ^{14}C analysis showed that the amount of ^{14}C-labeled TNT that remained on the sediment after extraction was about 7%, which cannot be accounted for by analytical error alone. Either irreversible sorption or transformation of the TNT is taking place on the sediment. No evidence of transformation products from the TNT was found after 240 hours, as was the case in earlier isotherms. A cell count showed that the flasks containing TNT and sediment were sterile after 240 hours. Since the products found earlier did not appear in the flasks containing sediment only, they must have been a product either of biotransformation or reaction of TNT with the sediment. The recovery of TNT after 240 hours, when no attempt at sterilization was made, was 30-40%, 60% when $HgCl_2$ was added, and 93% when $HgCl_2$ was added and the sediment was autoclaved. Therefore, it is most likely that losses of TNT were due to biodegradation and irreversible sorption, but this does not exclude reaction with the sediment as a possible means of TNT losses.

d. Summary

A summary of the partition coefficients (both K_p and K_{oc}) is presented in Table 18 for all the sorption and desorption isotherm measurements. The error associated with the data for the first desorption isotherm measurement was very high because only very small amounts of TNT were measured, and therefore no value of K_p or K_{oc} is reported.

Table 17

DISTRIBUTION AND RECOVERY OF TNT IN THIRD ISOTHERM

	0 h				240 h				
	Supernatant µg (%)	Sediment Extract µg (%)	Sediment[a] µg (%)	Total µg (%)	Supernatant µg (%)	Sediment Extract µg (%)	Sediment µg (%)	Total µg (%)	K_p µg/g/µg/ml
C^{14}	1.87 (92)	0.31 (15)	0 (0)	2.18 (107)	1.15 (55)	0.74 (35)	0.15 (7)	2.04 (97)	43
EPLC	95.5 (89)	17 (16)	-- --	112.5 (105)	65.0 (56)	42.4 (37)	-- --	107.4 (93)	36

[a]Concentration remaining in the sediment after extraction with ethyl acetate.

Table 18

SUMMARY OF TNT SORPTION DATA

Experiment	K_p	K_{oc} (TOC = 3.3%)
Screening isotherm (after 15 h)	30	910
Sorption isotherm #1 (after 168 h)	63	1900
Sorption isotherm #2 ($HgCl_2$ added, after 144 h)	42	1300
Desorption isotherm #2 ($HgCl_2$ added, after 48 h)	84	2500
Sorption of ^{14}C ($HgCl_2$ added, autoclaved, after 240 h)		
Determined by HPLC	35	1100
Determined by ^{14}C analysis	43	1300
Average sorption partition coefficients (Screening isotherm excluded)	53 ± 20	1600 ± 600

7. Model Simulations

a. Adjusted Rate Constants

The biodegradation rate constant for TNT was estimated to be 1.0×10^{-2} day^{-1} at a cell level of 10^6 cells ml^{-1}. This value was assumed for the 3 water bodies under both high and low flow conditions. The photolysis rate constant ranged from 28 to 40 $days^{-1}$, depending on the flow conditions, and photolysis therefore represented the most dominant transformation process acting on TNT. The sediment partition coefficient was low for all 3 water bodies (estimated to be 40) and did not have a significant effect on the model simulations. Tables 19 and 20 list the adjusted rate constants for each compartment of the three water bodies investigated.

b. Simulation Results--Waconda Bay

Waconda Bay was compartmentalized as shown in Figure 23 and discussed in Appendix B. Monitoring data (Sullivan et al., 1977) show that TNT entering the bay travels along the west bank because the intrusion of Tennessee River water causes the plant effluent to move along the west side of the bay.

In this simulation study, the intrusion of Tennessee River water (as discussed in Appendix B in the section on flow characteristics in Waconda Bay) was assumed with a clockwise circulation pattern. With these assumptions, one may speculate that the returning flows will decrease as the intruding water moves up to the head of the bay, with the result that not only will TNT be transported faster down to the lower bay, but also that the bay waters will be more mixed. Theoretically, increased mixing of the bay water will result in increased chances of TNT being brought up to the water surface and lead to an increase in the TNT photolysis rate. Furthermore, since the bay water is relatively clear and moderately deep, TNT will photolyze fairly rapidly on a sunny day.

Figure 24 shows the TNT distribution in Waconda Bay at a plant discharge rate of 220 liters sec^{-1} and 1100 liters sec^{-1}. Because the amount of plant effluent is much smaller than the amount of intruding water from the Tennessee River, the projected TNT concentrations for

Table 19

COMPARTMENT PARAMETERS USED IN THE SIMULATION OF TNT TRANSFORMATION IN THE HOLSTON AND NEW RIVERS

Water Body (Compartments)	Depth (m)		Suspended Sediments (mg liter^{-1})	Photolysis Rate Constant (Day^{-1})		Biotransformation Rate Constant (Day^{-1})
	High Flow	Low Flow		High Flow	Low Flow	
Holston River (20)	1.2	0.6	20	30	35	1×10^{-2}
New River (28)	3.5	2.0	20	30	40	1×10^{-2}

Table 20

COMPARTMENT PARAMETERS USED IN THE SIMULATION OF TNT TRANSFORMATION IN WACONDA BAY

Compartment Number	Depth (m)	Suspended Sediments (mg liter^{-1})	Photolysis Rate Constant (Day^{-1})	Biotransformation Rate Constant (Day^{-1})
1	0.6	15	35	1×10^{-2}
2	0.9	15	35	1×10^{-2}
3	0.9	15	35	1×10^{-2}
4	1.5	15	32	1×10^{-2}
5	2.4	10	30	1×10^{-2}
6	3.0	10	28	1×10^{-2}
7	3.0	10	28	1×10^{-2}
8	3.0	10	28	1×10^{-2}
9	3.0	10	28	1×10^{-2}
10	3.0	8	30	1×10^{-2}
11	3.0	8	30	1×10^{-2}
12	3.0	8	30	1×10^{-2}

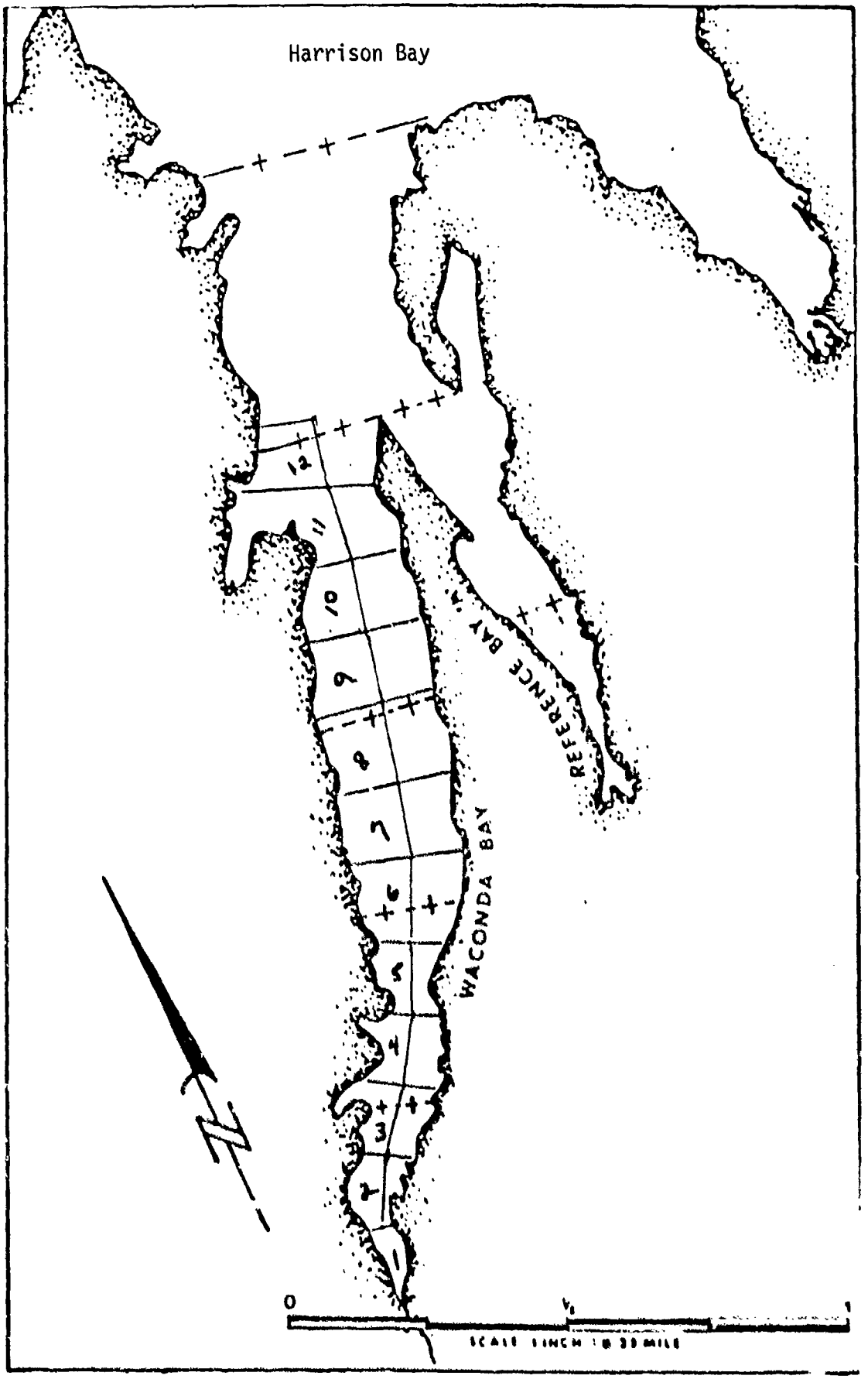


FIGURE 23 COMPARTMENTALIZATION OF WACONDA BAY FOR SIMULATION STUDY
COMPARTMENT VALUES RANGE FROM 16-120 x 10^2 m^2

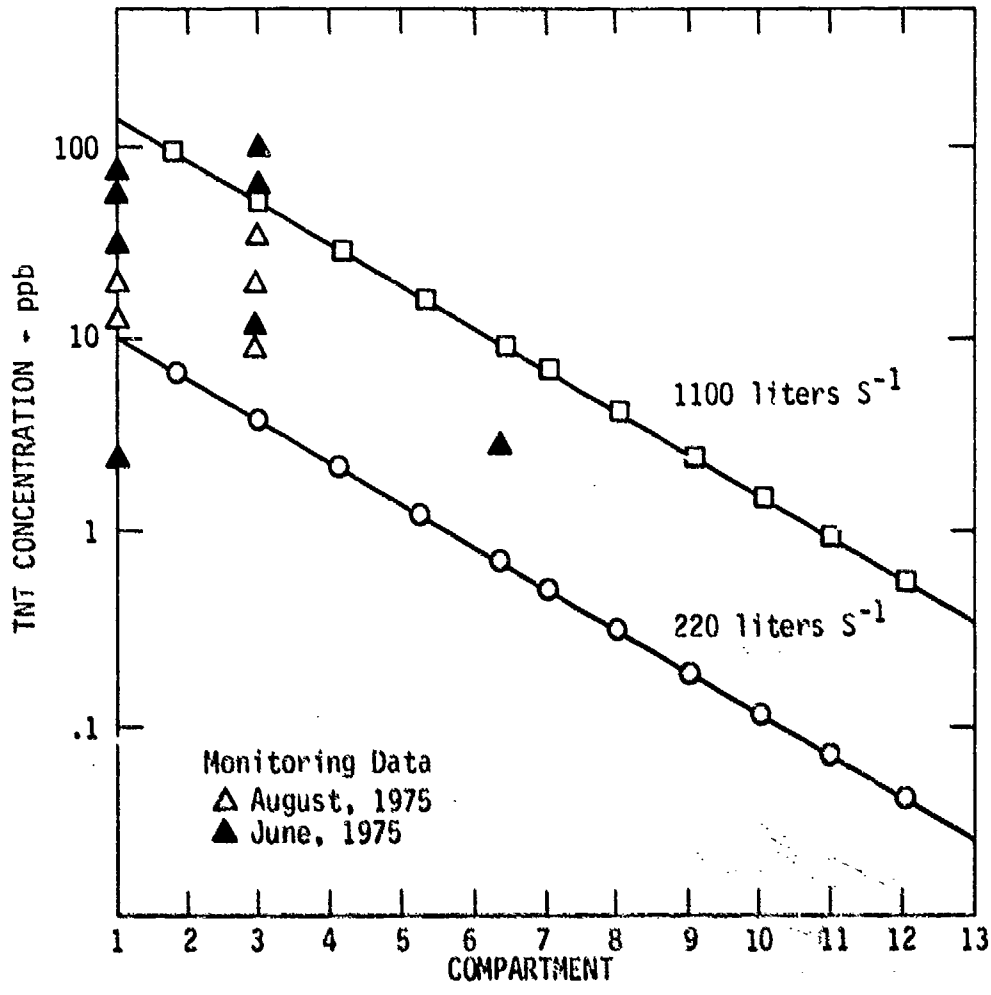


FIGURE 24 PROJECTED TNT CONCENTRATIONS OF THE PLANT EFFLUENT AT 220 LITERS S^{-1} AND 1100 LITERS S^{-1} AND ACTUAL TNT CONCENTRATIONS OF SAMPLES TAKEN FROM WACONDA BAY DURING 1975

both discharge rates decrease in parallel as the water moves toward the lower bay. At the head of the bay, the simulation results show that photolysis is as important as dilution from the intruding water. However, dilution increases in importance when TNT reaches the middle of the bay. The simulation results also indicate that TNT concentrations in the middle of the bay are already below 1 ppb.

In a 1975 survey on TNT in Waconda Bay, TNT was found mainly in the area corresponding to compartments 1 to 4 (Sullivan, 1977), but trace amounts of TNT were detected beyond compartment 12. This could be because mixing in the bay was poor as well as because there was less dilution than expected from the intruding water. Furthermore, the meteorologic conditions during the survey period may not have favored photolysis. Because of those reasons, TNT may be found at some areas in the bay.

The residence times of the plant effluent in Waconda Bay, based on 220 liters^{-1} and 1100 liters^{-1} discharge rates and 4.4×10^4 liters sec^{-1} of intruding water from the Tennessee River, were estimated to be about one-half day. It is likely that the plant effluent will take even longer to reach Harrison Bay. If this is true, TNT will be almost completely photolyzed before reaching the lower bay.

Laboratory studies revealed that biotransformation of TNT is much slower than photolysis. However, the biotransformation rate may become competitive when the microbial population is large and the sky is overcast. In general, the microbial population in an estuary is expected to be slightly larger than that in a moving water stream due to the higher organic content of estuaries. Moreover, the head of Waconda Bay constantly receives organic wastes from the Volunteer plant and therefore a microbial population larger than normal microbial populations may be expected. At the microbial population of 10^7 cells ml^{-1}, the half-life for biodegradation of TNT is still in the range of 1 day. In conclusion, TNT discharged from the Volunteer plant will be photolyzed

readily in Waconda Bay. The TNT concentration expected at the head of the bay will be about 10 ppb. TNT concentrations at the discharge point can reach as high as 0.1 ppm. However, it is reasonable to believe that TNT concentrations in the lower bay will be1 ppb or less.

c. Simulation Results--Holston River

The Holston River is shallow and wide and was compartmentalized according to the scheme shown in Figure 25. The average depth of the river ranges between 1.2 and 1.8 meters in a mean flow period. The mean depth may reduce to 0.6 meter during a low flow period. If the TNT loading is assumed to be constant, the dilution ratio during the mean flow is about 5 times greater than that during the low flow period.

Figure 26 shows the TNT concentrations in the Holston River in both low and mean flow periods. TNT concentrations above 10 ppb were predicted near the discharge point during the low flow period. However, TNT concentrations were projected to fall below 1 ppb in both low and mean flow periods at compartment 8, which is close to the location of sampling point 4'. Monitoring data collected at sampling point 4' indicated that TNT was not detected. Although no detailed information on TNT detectable levels were discussed in the monitoring program report (Sullivan et al., 1977b) the TNT concentration at sampling point 4' was probably at sub-ppb level. The TNT concentration in the Holston River at the boundary of the plant was projected to be about 0.1 ppb.

Segregation in the Holston River from the confluence point of North and South Fork Rivers had been observed at flows slightly below the mean flow (Sullivan, 1977b). Segregation will probably be greater during a low flow period. Segregation means that the river waters are not mixed well, in which case the dilution ratio is not as high as expected. Therefore, high local TNT concentrations can be observed at the north bank of the Holston River when segregation becomes apparent. Moreover, a decrease in TNT concentration because of dilution cannot be fully recognized until complete dispersion is established several kilometers downstream from the discharge point.

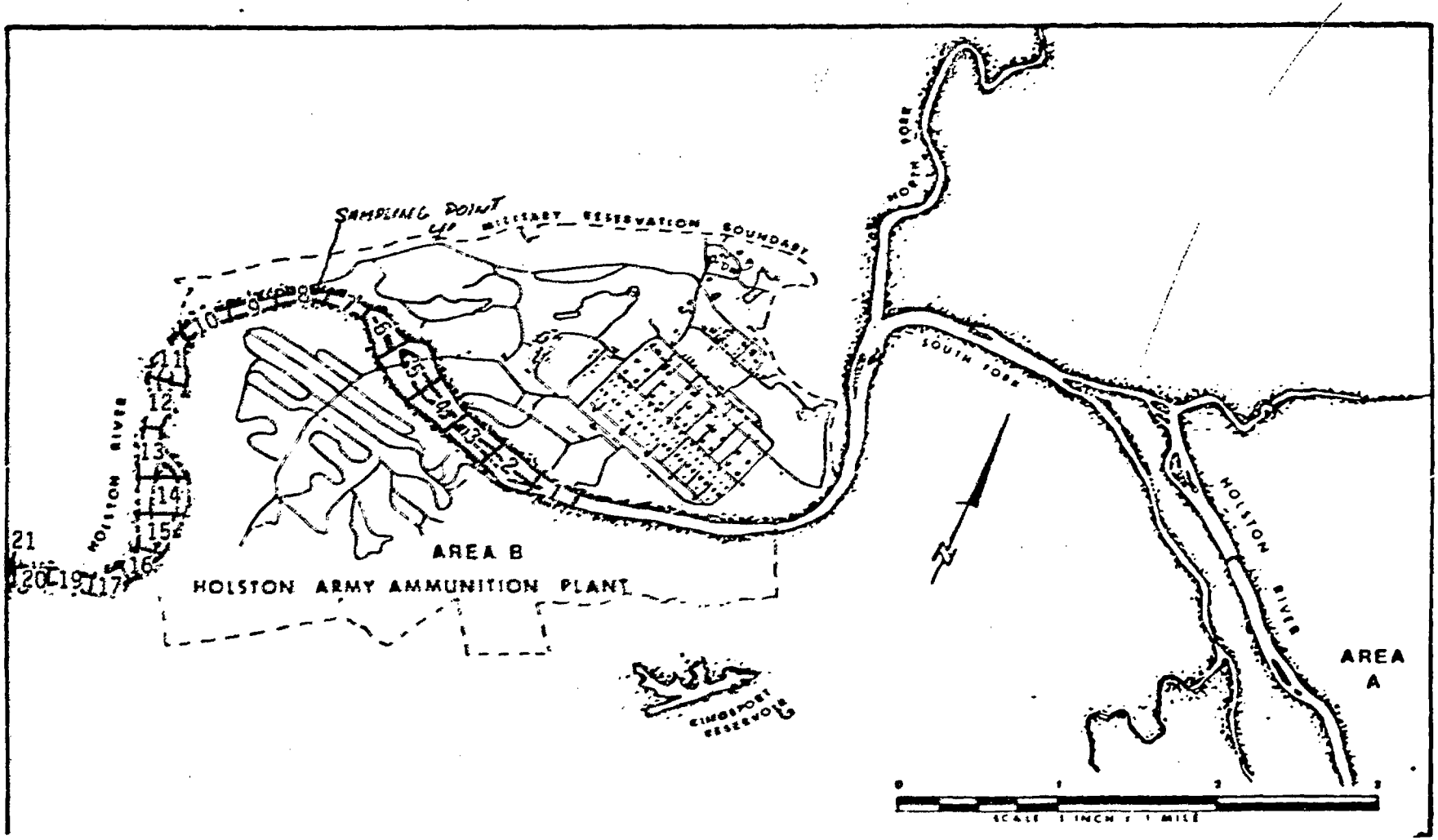


FIGURE 25 MAP OF HOLSTON AAP STUDY AREA AND COMPARTMENTALIZATION OF THE HOLSTON RIVER EACH COMPARTMENT IS 0.4 Km IN LENGTH

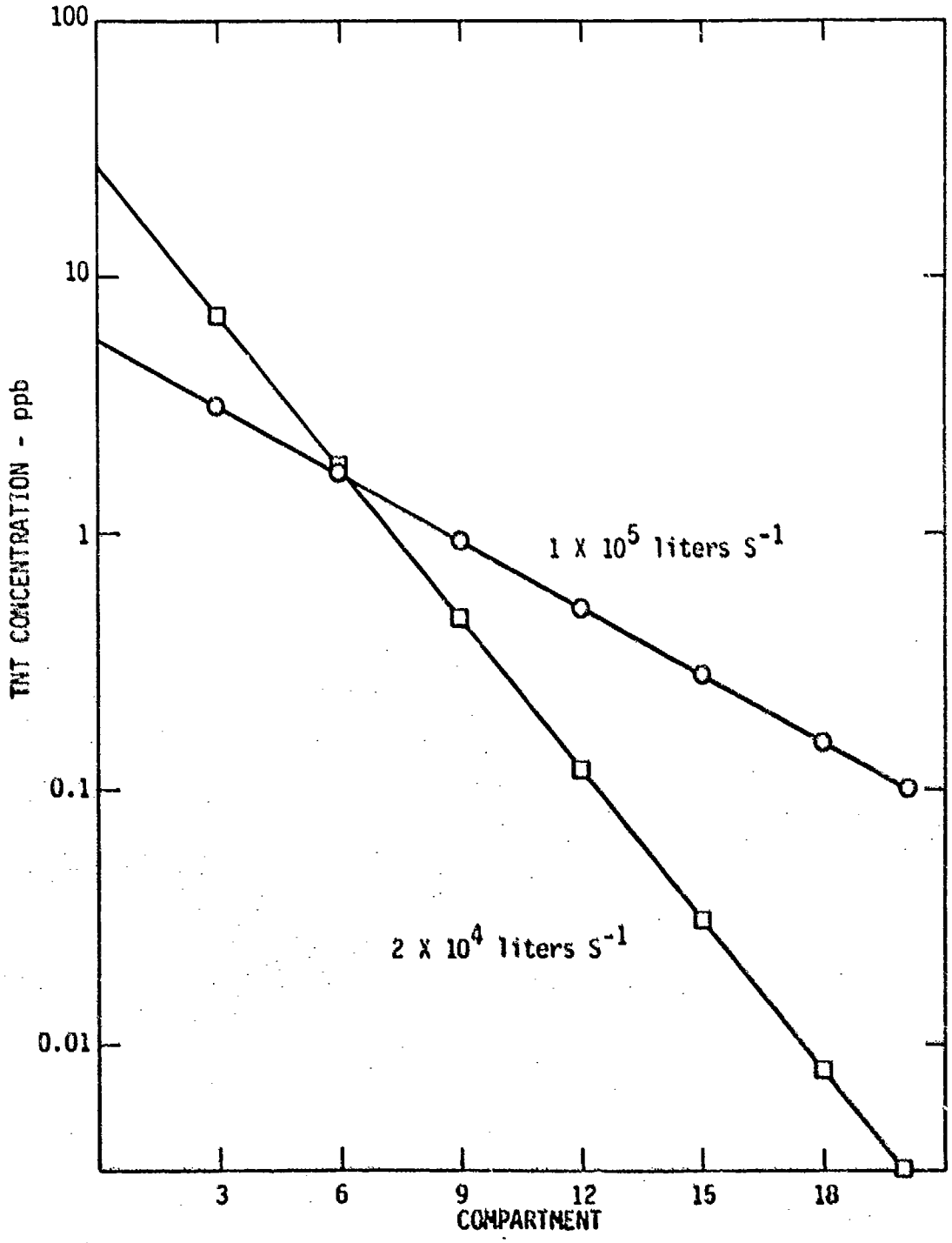


FIGURE 26 TNT SIMULATION RESULTS IN THE HOLSTON RIVER

d. Simulation Results--New River

The New River was compartmentalized according to the scheme in Figure 27. Since TNT wastewaters emanate from Stroubles Creek, initial dilution of TNT wastes occurs in compartment 7.

The in-stream flow of 2.0×10^2 liters s^{-1} in Stroubles Creek represents the RAAP plant waste effluent and the cooling water discharge from the plant. This flow is approximately 2% of the New River flow during the low flow period and 0.1% of the New River flow during the mean flow period. Therefore, the dilution factor during the mean flow period should be at least 20 times greater than that during the low flow period. Furthermore, the average photolysis rate during the mean flow period should be slower than that during the low flow period because the water is deeper and less transparent from more suspended solids.

Figure 28 shows the TNT concentration profile in the New River during both low flow and mean flow periods. The simulation results show that the 4.5 ppb of TNT concentration at the confluence point (compartment 7) at the low flow period is much higher than the 0.5 ppb level at the mean flow period. Approximately 40% of the TNT is photolyzed every 0.5 kilometer during the low flow period. In contrast, only 10% of the TNT is photolyzed in the same river span during the mean flow period. The TNT concentration at the end of the 14-kilometer river reach was projected to be about 80 ppt during the mean flow period in comparison to less than 7 ppt at the low flow period.

In conclusion, TNT concentration was projected to be high at the confluence point during the low flow period. However, the TNT would soon be photolyzed, and levels below 0.1 ppb are expected within short distances from the discharge point.

8. Conclusions

TNT was found to photolyze rapidly in natural waters, leading to environmental half-lives of 3 to 22 hours. The photolysis rate is

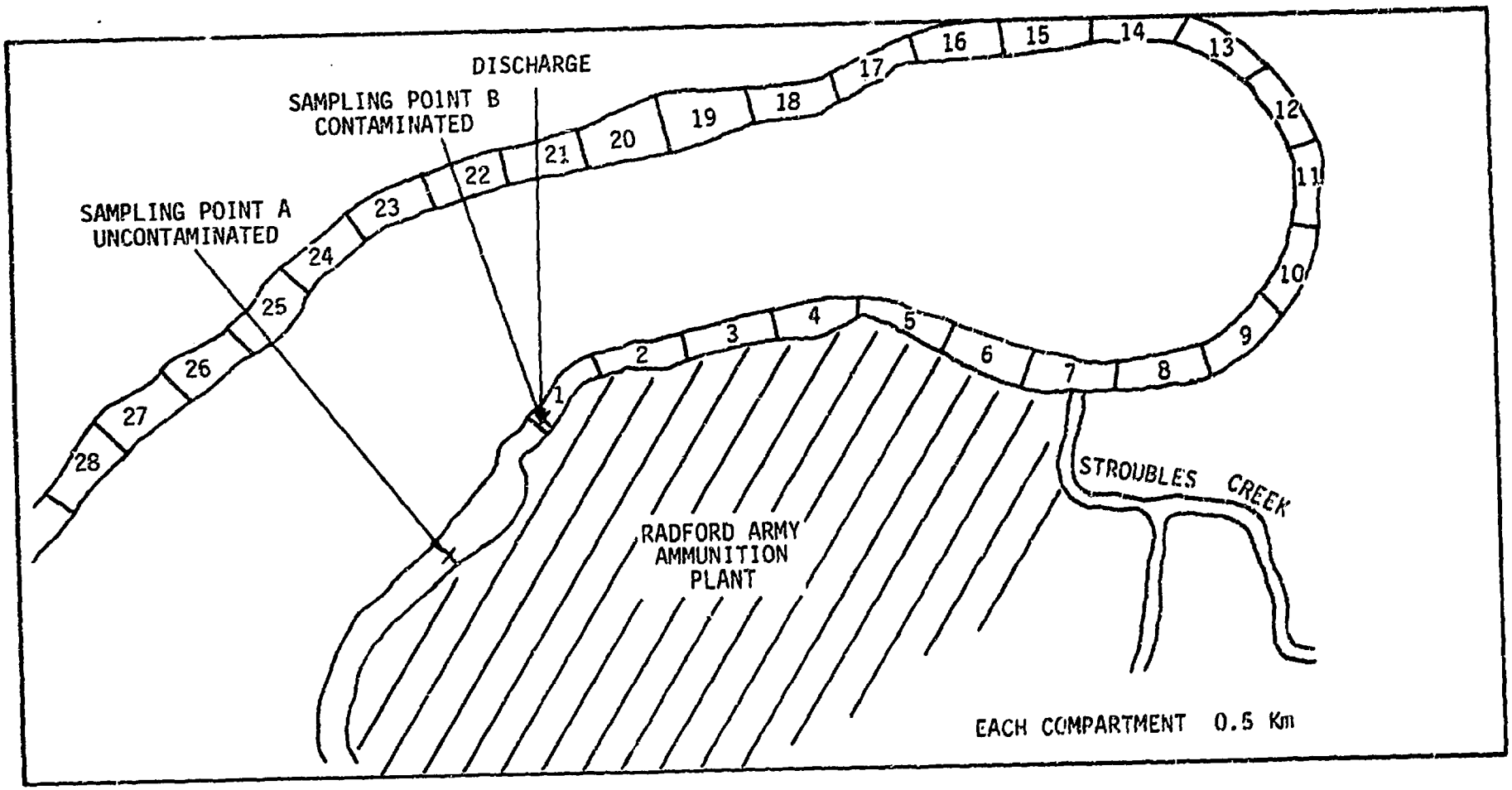


FIGURE 27 COMPARTMENTALIZATION OF NEW RIVER

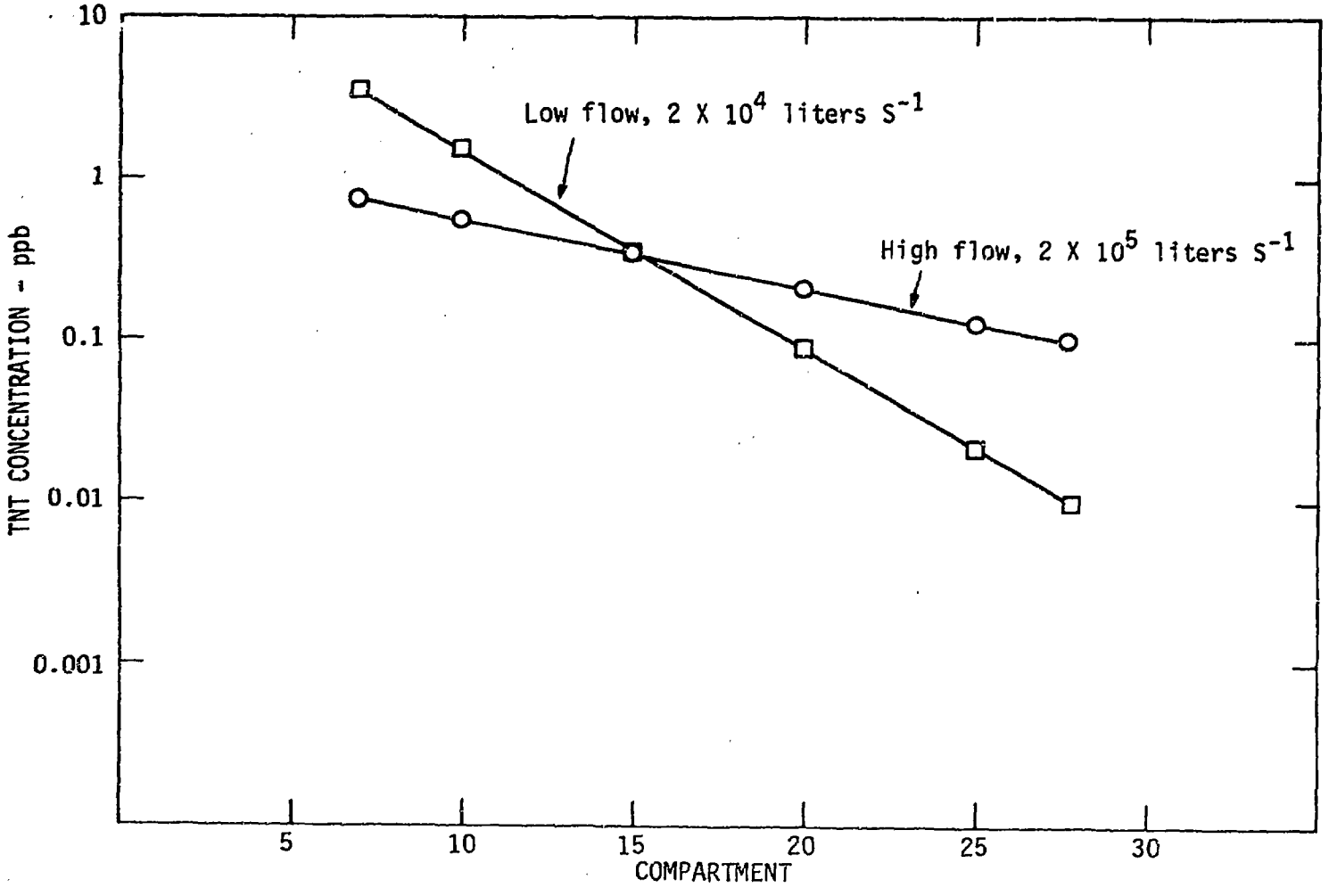


FIGURE 28 TNT SIMULATION RESULTS IN THE NEW RIVER

accelerated by the natural substances in a water body and was found to vary by a factor of up to 60 compared to that in distilled water. Triplet sensitization is thought to be the mechanism for acceleration.

Biotransformation is expected to be a slow process in natural waters containing cell populations of $\approx 10^6$ cells ml^{-1}. Half-lives ranging from 8 to 25 days were observed after 13- to 40-day acclimation periods. The acclimation periods became less important with high cell populations. At 10^9 cells ml^{-1}, half-lives ranging from 4.0 to 5.6 hours were observed with no acclimation period and standard ATCC organisms were able to transform TNT. The primary mode of metabolism is nitro-group reduction, and no aromatic ring rupturing was observed in ring-labeled ^{14}C-TNT studies. Thus, it is expected that TNT will not be mineralized by microbes in the environment.

Sorption to sediment and biomass was found to be small and will not control concentrations of TNT in aquatic systems. Therefore, the environmental half-life, $t_{\frac{1}{2}}$ (envir), of TNT can be estimated from equation 25

$$t_{\frac{1}{2}}(\text{envir}) = \frac{0.692}{k_p} \quad , \tag{25}$$

where k_p is the photochemical rate constant for a particular water body.

Model simulations of TNT-receiving water bodies show rapid loss of TNT by photolysis. This loss is larger during periods of low flow because of the longer time required to traverse two points and greater exposure to sunlight afforded in shallow water. Under mean flow conditions, the initial TNT dilution is greater than it is under low flow conditions, but further downstream, TNT concentrations may exceed those under low flow conditions. Thus, TNT will traverse the greatest distance from the discharge point under high flow conditions during cloudy days.

B. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

1. Background

RDX has been reported to photolyze by several investigators (Kubose and Hoffsommer, 1977; Spanggord et al., 1978). These findings indicated that this process should be studied in detail. The Phase I literature review revealed a paucity of information on the biotransformation of RDX, which meant that this process had to be investigated through initial screening studies and followed by detailed studies if warranted. Sediment and biomass sorption were considered to be nonsignificant fate processes for RDX based on reports from Syracuse Research Corporation (1977); however, because photolysis and biotransformation processes were expected to have small rate constants, sorption partitioning may become a competitive process if environmental half-lives were large. Therefore, screening studies to measure sorption partition coefficients were performed.

2. Photochemistry

Photolysis of RDX in distilled water and in two filter-sterilized natural water samples followed first-order kinetics when RDX was photolyzed in sunlight or at 313 nm. The photolysis rate constants for these experiments are listed in Table 21 along with the sunlight photolysis half-lives observed. The UV absorption spectral data listed in Table 22 show that RDX has only a small absorption in the solar spectral region. The sunlight photolyses of RDX in 2 natural waters and distilled water were conducted simultaneously during winter from 3 January to 21 January 1980. The weather was mostly cloudy and rainy during this period, with temperatures ranging from approximately -2 to 18°C. Filter-sterilized Holston River and Searsville Pond waters were used for the photolyses; 0.5 ppm RDX was already present in the Holston River water before RDX was added for the photolysis experiments.

The data in Table 22 show that the photolysis of RDX is only slightly accelerated in the Searsville Pond water over the photolysis observed in distilled water solutions. The photolysis of RDX in the

Table 21

PHOTOLYSIS RATE CONSTANTS FOR 1.3 ppm RDX IN NATURAL AND DISTILLED WATERS[a]

Water	Photolysis Rate Constant at 313 nm $k_p \times 10^5$ (s^{-1})	Photolysis Rate Constant in Sunlight $k_p \times 10^7$ (s^{-1})	Sunlight Photolysis Half-life (Days[b])
Distilled water	3.60 0.04	6.3 ± 0.2	13
Holston River[c,d]	ND[e]	5.8 ± 0.2	14
Searsville Pond[c]	ND	8.9 ± 0.2	9

[a] 1.3 ppm = 5.85×10^{-5} M RDX.

[b] Half-life is in terms of 24-hour days.

[c] Natural waters were filtered through a 0.45-μ filter.

[d] Holston River water collected on 7 August 1979 had 0.5 ppm RDX present.

[e] ND = not determined.

Table 22

ABSORPTION SPECTRUM OF RDX

Wavelength (nm)	Absorption Coefficient ($M^{-1}cm^{-1}$)[a]		
	ε[b]	ε[c]	ε_{ave}
297.5	160	157	159
300.0	150	140	145
302.5	113	132	123
305.0	95	99	97
307.5	85	72	78
310.0	57	64	60
312.5	47	59	53
315.0	37	40	39
317.5	28	31	30
320.0	28	22	25
323.1	19	17	18
330.0	9	7	8
340.0	0	1	0
350.0	0	0	0

[a]Absorption spectrum of RDX was measured at a higher concentration in 1-cm cells and at a lower concentration in 10-cm cells to check the reproducibility of absorption coefficients.

[b]For 23.6 ppm RDX (1.06×10^{-3} M) in 10% acetonitrile/90% water, 10-cm cell.

[c]For 101.3 ppm RDX (4.56×10^{-3} M) in 100% acetonitrile, 1-cm cell.

Holston River occurred at about the same rate as in distilled water; the difference in the rate constants is almost within experimental error, but may also be due to a slight light-screening effect of the natural water. From these experiments, it appears that indirect photolysis of RDX due to natural substances will not be important in aquatic environments.

The reaction quantum yield for photolysis of RDX in distilled water is 0.16 at 313 nm. Using this quantum yield and the absorption spectrum in Table 21, we calculated the half-lives for RDX photolysis in shallow, distilled waters as functions of latitude and season according to the method of Zepp and Cline (1977). These data are given in Table 23.

Table 23

ANNUAL VARIATION OF RDX PHOTOLYSIS HALF-LIFE IN SUNLIGHT IN PURE WATER[a]

	Latitude N		
	20°	40°	50°
Summer	1.1	1.2	1.3
Fall	1.4	2.6	4.4
Winter	1.8	5.0	12.5
Spring	1.2	1.5	2.0

[a]Half-lives are in 24-hour days.

The measured half-life of 13 days during winter in Menlo Park (at 37° latitude) is over twice that calculated from the quantum yield and absorption spectral data. This result may be explained in part by the overcast weather during the outdoor experiment, because the calculated half-life uses sunlight intensity and wavelength distribution data for clear days. A subsequent sunlight photolysis experiment conducted from 24 March to 28 March 1980, during clear weather (temperature

range, 5°C to 20°C), resulted in a measured half-life of 1.8 days, which is in good agreement with the 1.5 day half-life calculated for spring season at 40° latitude.

3. RDX Photoproducts

The photolysis experiments with RDX were followed by HPLC analysis of the photolyzed solutions by direct aqueous injection and by HPLC analysis of the ethyl acetate extracts of the photolyzed solutions. No photoproducts were observed in these profiles using UV detection at 254 nm. In an attempt to identify an N-nitroso analog of RDX reported by Kubose and Hoffsommer (1977) to result from the photolysis of RDX in acidic aqueous solution, the ethyl acetate extract of a photolyzed RDX solution was analyzed by HPLC coupled to a thermal energy analyzer detector (TEA), a detector with high specificity and sensitivity for N-nitroso compounds. Again, no photoproducts were observed in the HPLC profiles.

Formaldehyde was identified as a photoproduct as a 2,4-dinitrophenylhydrazone derivative by GC and confirmed by GC/MS. Also, nitrate and nitrite were observed as photoproducts by HPLC using an ion-exchange column and UV detection at 210 nm. In one experiment, the formation of formaldehyde, nitrite, and nitrate followed the curves shown in Figure 29. The formation of formaldehyde parallels the formation of nitrite. However, the mechanism for formaldehyde production is not clear from these data because a material balance of reactants and products could not be obtained.

4. Biotransformation

a. Screening Test

The RDX biotransformation screening test was initiated with Holston River water. RDX (10 ppm) in river water collected in July was not significantly degraded during 78 days of incubation. The same result was obtained using water collected in August during 73 days of incubation. The addition of 30 ppm yeast extract in river water did not significantly affect the disappearance of RDX.

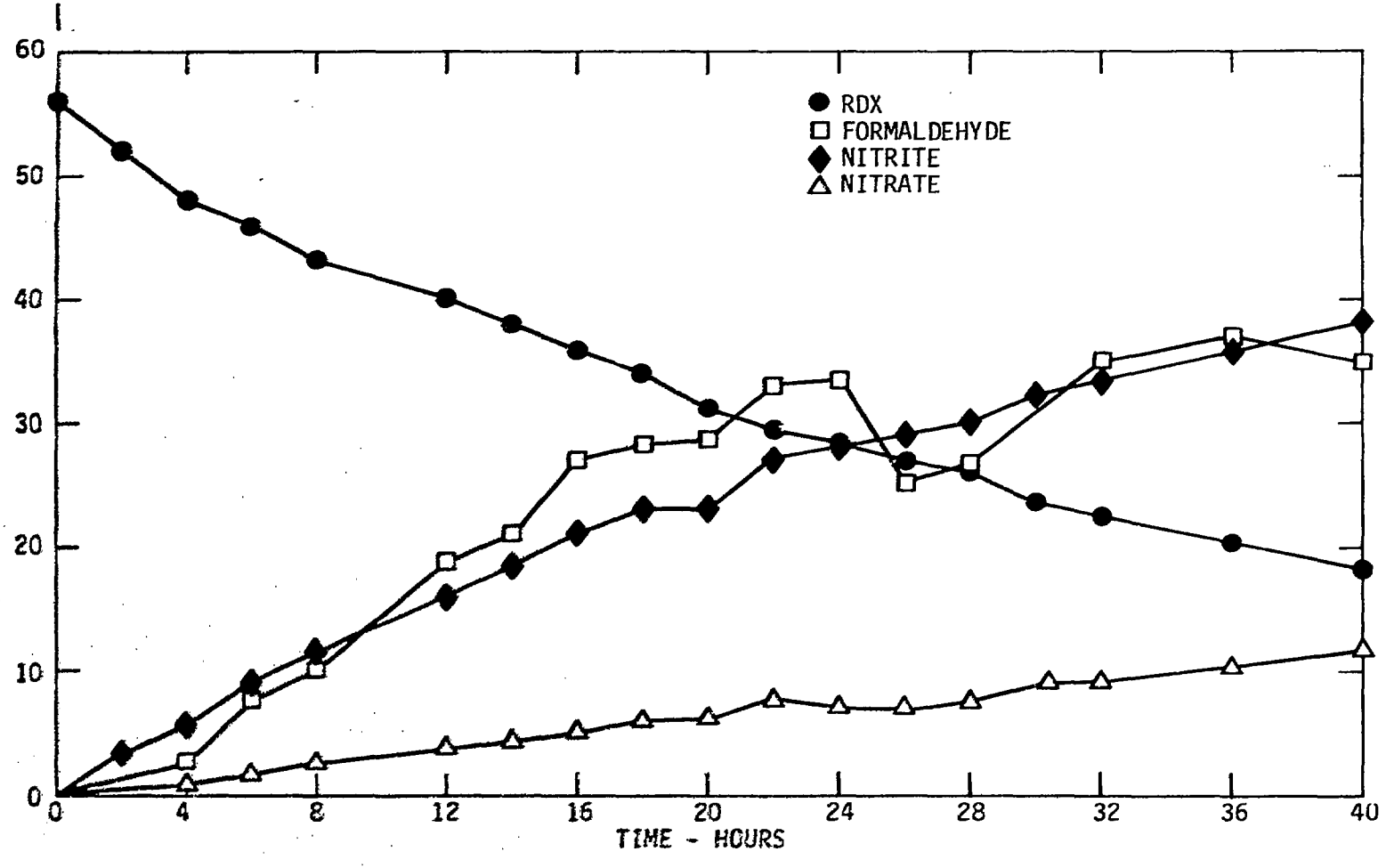


FIGURE 29 PHOTOLYTIC DEGRADATION OF RDX IN DISTILLED WATER IN SUNLIGHT AND THE FORMATION OF FORMALDEHYDE, NITRITE, AND NITRATE

In August water to which 1% Holston River sediment was added, the initial RDX concentration (10 ppm) remained unchanged for 20 days, then reduced to 4 ppm from day 20 to day 36. The RDX concentration remained the same for another 2 weeks of incubation. Bacterial cell plate count was 6.3×10^6 cells ml^{-1} on day 45. In the water without sediment, the bacterial plate count was 4.0×10^5 cells ml^{-1}.

Aliquots of water from the bottle with 1% sediment were transferred into flasks containing basal-salts medium and 10 ppm of RDX, RDX plus 1% sediment, or RDX plus 100 ppm Trypticase Soy broth, or containing RDX alone plus a boiling water extract of sediment (equivalent to 2% sediment). No RDX transformation was observed in any of the flasks after 27 days of incubation.

Water from a local eutrophic pond was collected and RDX biotransformation screening was also initiated with 10 ppm of RDX only, RDX plus 1% local pond sediment, and RDX plus 1% Holston River sediment. The RDX concentration in pond water alone or pond water plus pond sediment did not change significantly during 90 days of incubation. The RDX concentration in pond water plus Holston River sediment, however, had decreased to nearly 1 ppm at 76 days after 20 days of lag period with a half-life of 18 days. The bacterial plate count was 2.3×10^6 cells ml^{-1} on day 41. The microorganisms in this water were transferred to shaker flasks containing basal-salts medium and 10 ppm RDX alone, RDX plus 50 ppm of yeast extract, and RDX plus 1% Holston River sediment. No biotransformation was observed in any of the shaker flasks after 30 days of incubation. These data data suggested that RDX will be resistant to biotransformation under aerobic conditions.

Compared to the persistence of RDX under aerobic conditions, RDX was readily transformed anaerobically with small amounts of extra organic nutrients. RDX in Holston River water with 30 ppm yeast extract was transformed to less than 0.1 ppm within 10 days. In local pond water containing 10 ppm RDX and 50 ppm yeast extract, RDX decreased to < 0.1 ppm in 8 days. No significant RDX transformation

was observed in anaerobic flasks without added yeast extract. In the transferring flasks containing RDX and yeast extract, RDX disappeared in 2 to 3 days. The dissolved oxygen concentration was less than 1% of saturation in these flasks. These studies suggested that aerobic bacteria in a mixed culture may utilize the yeast extract to grow and consume oxygen to maintain anaerobic conditions. Studies were conducted to determine if yeast extract was needed as nutrients for RDX transformation or needed to maintain an anaerobic environment. The Holston River RDX-degrading anaerobes were inoculated into a flask with RDX and basal-salts medium without yeast extract, and N_2 gas was bubbled into the medium to remove dissolved oxygen before the inoculation and after each sampling instead of just flushing N_2 gas into the head space. In this flask, 50% of the RDX was transformed in 4 days and 80% was transformed in 12 days. When these organisms were incubated in another flask containing RDX as the only added organic nutrient, only 25% of the RDX was transformed in 20 days. These results showed that RDX anaerobic degradation depends on extra organic nutrients and that a cometabolic process is probably involved in the degradation.

To determine if the yeast extract functioned as a cosubstrate for cometabolism or only provided carbon nutrient to grow enough cell biomass for significant transformation, microorganisms were grown in 50 ppm yeast extract without RDX, and 4 ppm RDX was added after 2 days of incubation. RDX was not transformed after 19 days of further incubation. In a control flask initially containing 10 ppm RDX and 50 ppm yeast extract, RDX disappeared in 3 days. These results suggest that yeast extract and RDX must be present at the same time to achieve RDX transformation and the yeast extract serves as a cometabolic substrate.

Because RDX was not biodegraded in a short time under aerobic conditions, no detailed rate study was conducted.

b. Discussion

RDX is not readily biotransformed in water under aerobic conditions and is expected to be relatively persistent in aquatic environments. It will, however, biodegrade slowly in the presence of 1% Holston River sediment after a lag period. This phenomena was also reported by Sikka et al. (1980). The production of 80% of the theoretical amount of $^{14}CO_2$ from ^{14}C-RDX that they reported clearly showed that RDX can be mineralized by biotransformation. In these cases, the sediment may have (1) acted as a source of transformation microorganisms, (2) acted as a source of growth factor nutrients, (3) acted as a source of cometabolic substrate, or (4) provided a special physical environment, or combination of the above. The mechanism of sediment-enhanced transformation is not clear at this time, because further transferring of organisms in the acclimation phase failed to show biotransformation in our preliminary tests. In the initial screening test bottle, the bottle was gently aerated from a sparger, and the water was mixed 2 to 3 times a week. The transfer flasks were shaken on a shaker incubator. Because RDX can be biotransformed readily under anaerobic conditions with extra organic nutrient, extensive aeration, such as that found with shaker-flask incubation, may hinder RDX degradation.

Normally, river waters are aerobic and RDX will not biotransform in this environment. However, lakes or shallow waters have anaerobic environments that are rich in natural or synthetic organic material from aquatic organisms, land run-off, or wastewater. When eutrophic river water enters a lake (such as that created by a dam) or at some point where the river flow slows, there can be local anaerobic areas at the bottom of the waterbody. In these environments, RDX may be biodegraded. The extent to which this process affects the environmental fate of RDX is unknown.

5. Biosorption

A biosorption test was conducted with a mixture of viable washed cells of 4 ATCC test organisms. Preliminary tests showed that RDX was not biotransformed by biosorption test microorganisms during the testing period. The initial RDX concentration was 7 ppm and mixed cells concentration was 3.7 mg ml^{-1}. The average sorption coefficient (± S.D.) was 47 ± 2. This number is low and not environmentally significant.

6. Sediment Sorption

The screening isotherm for RDX was measured by preparing 2 flasks containing the compound and sediment, one flask containing only the compound, and 1 flask containing only sediment. The sediment used was a sterilized sample from the Holston River (upstream). To suppress biologic activity $HgCl_2$ was added to each flask. Table 24 lists the specific amounts of the substances added to each flask.

Table 24

MATERIALS USED FOR RDX SORPTION EXPERIMENTS

Material	Flask 1	2	3	4
H_2O (ml)[a]	100	100	100	100
RDX Solution (ml)	100	100	100	--
Sediment (g)	--	10	10	10
Saturated $HgCl_2$ Solution (drops)	10	10	10	10

[a]Volume is total volume of H_2O plus sediment.

Samples were taken at the beginning of the experiment and after approximately 1 week. The samples were centrifuged and the supernatant and sediment separated. The supernatant was analyzed directly by HPLC. The sediment was extracted with ethyl acetate before HPLC analysis. The results of the isotherm are summarized in Table 25.

Table 25

RDX DISTRIBUTION IN SEDIMENT SORPTION EXPERIMENTS AND CALCULATED K_p and K_{oc} VALUES

Time (h)	Component Material	Flask 1	2	3	4
0	RDX in supernatant (µg/ml)	10.1	8.01	7.43	0
	RDX in sediment (µg/g)	--	lost	31.1	--
	K_p	--	--	4.2	--
	K_{oc}	--	--	127	--
168	RDX in supernatant (ug/ml)	9.18	9.15	10.6	0
	RDX in sediment (µg/g)	--	50.1	15.1	--
	K_p	--	5.5	1.4	--
	K_{oc}	--	167	42	--

The sediment sorption partition coefficient, K_p, and the sediment sorption coefficient based on organic carbon, K_{oc}, are extremely low, indicating that sediment sorption will not lead to significant RDX loss in the aquatic environment.

7. Model Simulations

a. Simulation Scenarios--Holston River

Two flow rates of 2×10^4 liters s^{-1} and 10^5 liters s^{-1} representing low and mean flow in the Holston River were selected for model simulations. Table 26 shows the laboratory-determined half-lives of RDX at various times of year and under various weather conditions. The photolysis half-lives varied from 1 to 14 days. Three scenarios

Table 26

LABORATORY ESTIMATES OF PHOTOLYSIS HALF-LIFE OF RDX

Time	Condition	Half-Lives (Days) Found	Half-Lives (Days) Estimated for 40° N Latitude
Spring	--	3-4	1.5
Summer	--	1.5	1.7
Fall	--	2	2.6
Winter	--	6-8	5.0
March	Clear, sunny	2	--
January	Rainy, cloudy	12.7	--

describing the worst case of low flow on a cloudy day and the best cases of low and mean flow on a sunny day were selected for model simulations. In the worst case, the photolysis rate constant for RDX was estimated to be 0.03 day^{-1}. In the best case on a sunny day, the photolysis rate constants for the low flow and the mean flow were estimated to be 0.23 day^{-1} and 0.17 day^{-1}, respectively. The low partition coefficient, which ranged from 5.5 to 1.4 ($\mu g\ g^{-1}\ \mu g^{-1}$ ml) indicates that an insignificant amount of RDX is transported through suspended solids. The partition coefficient of 5 ($\mu g\ g^{-1}\ \mu g^{-1}$ ml) was assumed for model simulations.

Although RDX could be biodegraded anaerobically, little RDX is expected to occur in the bottom sediment because the partition coefficient is low, thus making anaerobic degradation an insignificant fate process.

b. Simulation Results--Holston River

In the worst case, the simulated RDX concentrations remained constant with almost no changes within the 8.4-kilometer studied reach. Figure 30 shows the projected concentrations of RDX in the Holston River for low flow and mean flow at a high photolysis rate. Although the initial dilution as the waste stream discharged into the Holston

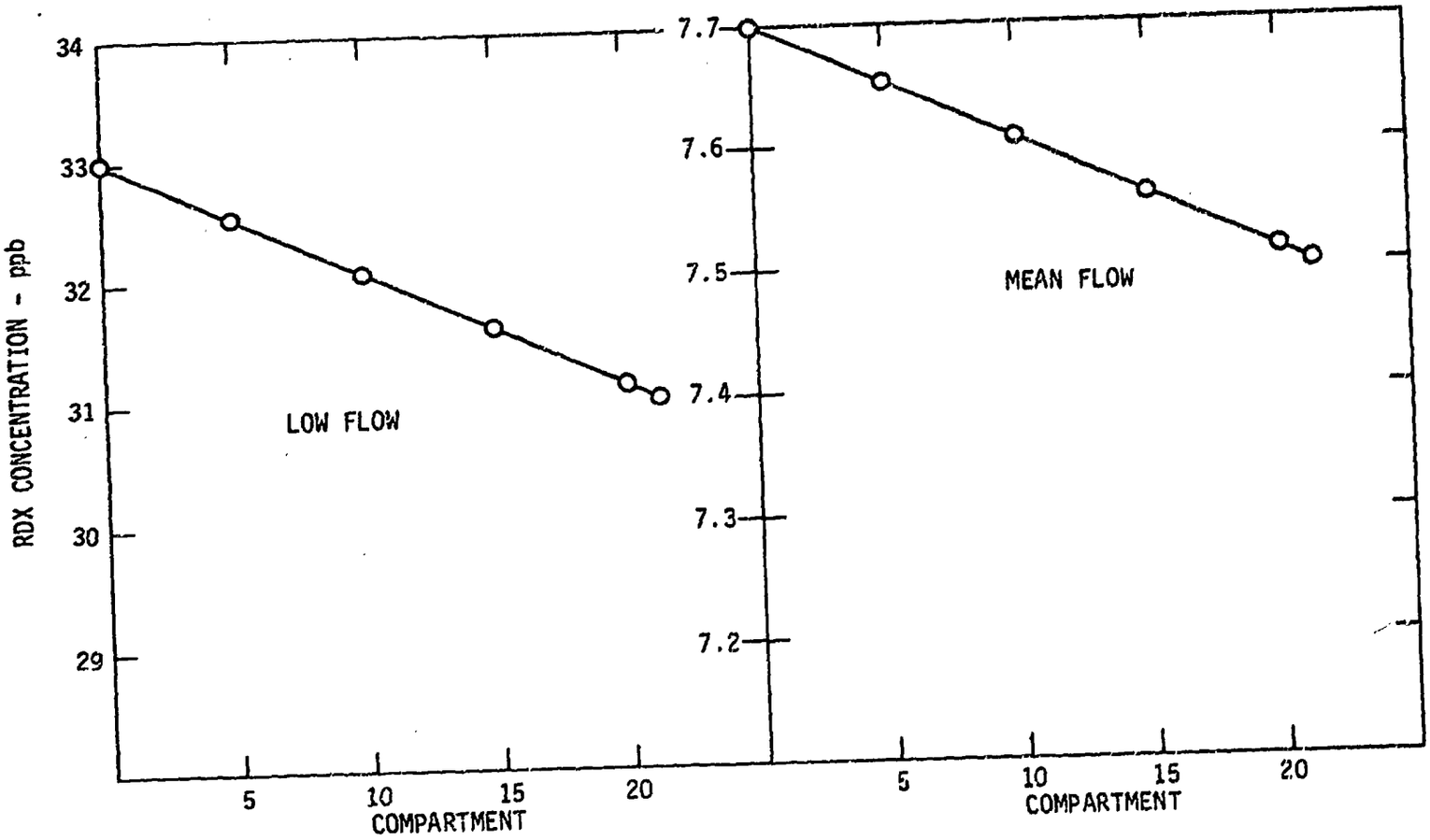


FIGURE 30 ESTIMATED CONCENTRATIONS OF RDX DURING LOW- AND MEAN-FLOW CONDITIONS ON A SUNNY DAY

River reduced the RDX concentration from 6,700 ppb in the waste stream to 33 ppb in the Holston River, the RDX concentration could be about 100 ppb in the area contiguous with the discharge point due to segregation of the river flow. Because RDX is persistent in the environment and unlikely to be deposited in the bottom sediment, dilution is the primary means of reducing the RDX concentration in the environment. The Holston River joins the Tennessee River at Knoxville, where the dilution factor is estimated to be at least 10. Therefore, the maximum RDX concentration in the Tennessee River at Knoxville was estimated to be about 3 ppb in the worst case.

In the case of RDX discharged on a sunny day into the Holston River during the low flow period, RDX was photolyzed within the studied reach, but not rapidly. About 6% of the discharged RDX was photolyzed at the end of 8.4 kilometers. If a constant photolysis rate and no further dilutions from other incoming flows were assumed, the RDX concentration in the Tennessee River at Knoxville was estimated to be approximately 1 ppb.

In the case of RDX discharged into the Holston River on a sunny day during the mean flow period, RDX was diluted to the level of 7.7 ppb at the discharge point and degraded approximately 2.6% at the end of the studied reach. Estimated RDX concentration in the Tennessee River at Knoxville was below 1 ppb.

8. Conclusions

RDX is a persistent chemical in the aquatic environment. The major environmental fate is expected to be dilution. The photolysis rate constant on a sunny day was estimated to be 0.23 day^{-1} in the Holston River, corresponding to a half-life of 3 days. The RDX discharged from the Holston AAP will remain in the Holston River for a long distance downstream from the discharge point. If the residence time of RDX in Cherokee Lake is several days, the RDX concentration in the Tennessee River will be expected to be much lower than that estimated. Therefore,

one can believe that the RDX concentration in the Tennessee River at Knoxville should be below the ppb level. Furthermore, segregation of the river flows at the confluence point of the North and South Fork of the Holston River can intensify the RDX contamination problem at the area contiguous with the discharge point. Better dispersion occurs as water flows downstream, but during the low flow period, segregation may occur for many kilometers, even beyond the plant boundary. This may lead to high RDX concentrations along the north bank of the river, particularly when RDX discharge concentrations are high.

C. 2,4-Dinitrotoluene (2,4-DNT)

1. Background

The Phase I literature review indicated that 2,4-DNT would undergo photolysis (Burlinson and Glover, 1977; Spanggord et al., 1978), biotransformation (McCormick et al., 1978), and volatilization (Spanggord et al., 1978), all of which could lead to loss of 2,4-DNT from the aqueous environment. Although we predicted that sediment sorption would not be a significant fate process, screening studies were performed to verify this prediction.

2. Photochemistry

The photolysis of DNT was similar to that of TNT discussed in Section IV-A, showing an acceleration of the photolysis rate with time in distilled water due to transformation products and exhibiting more rapid photolysis in several natural water samples than in distilled water. The rate constants for photolysis of DNT in distilled water were estimated by fitting the initial changes in DNT concentration as a function of time before the rate acceleration to the first-order kinetics equation in a manner similar to that used for the TNT data.

The rate constants for photolysis of DNT in air-saturated distilled water and in 3 filter-sterilized natural waters are given in Table 27; the rate constant for photolysis of DNT in the Waconda Bay water was

Table 27

PHOTOLYSIS RATE CONSTANTS FOR 1.0 PPM 2,4-DNT IN NATURAL AND DISTILLED WATERS[a,b]

Water	Photolysis rate Constant at 313 nm $k_p \times 10^5$ s^{-1}	Photolysis rate Constant in Sunlight $k_p \times 10^5$ s^{-1}	Sunlight Photolysis Half-life, hours
Distilled water			
Air-saturated	0.79 ± 0.08	0.47 ± 0.1	43
Argon-purged	2.4 ± 0.2	--	--
Holston River	10.2 ± 0.3	7.4 ± 0.3	2.7
Waconda Bay	5.3 ± 0.4	2.0 ± 0.2	9.6
Searsville Pond	6.6 ± 0.1	5.2 ± 0.1	3.7

[a] Photolysis rate constants for distilled water and Waconda Bay obtained from initial concentration data before rate acceleration (see text).

[b] 1.0 ppm = 5.6×10^{-6} M.

also calculated using the initial concentration data points because the DNT photolysis products gave an accelerated rate that exceeded the accelerating effect of the natural water itself. The UV absorption spectrum data listed in Table 28 show that DNT absorbs strongly in the solar spectral region.

The data in Table 27 also show that the rate constant in argon-purged distilled water is approximately 3 times larger than that in air-saturated water. This difference is identical to that found for TNT, and presumably occurs because oxygen quenches an excited triplet state. Although no detailed photochemical studies were performed to determine the mechanism of DNT photolysis, the photolysis of DNT probably occurs by a triplet mechanism similar to that suggested for TNT, and substances present in natural waters probably serve as triplet sensitizers.

No product studies were conducted for the photolysis of DNT. Because Burlinson, 1978, indicates that the photolysis of DNT and TNT give similar types of products, and because the photolyses of DNT and TNT have parallel reactivity in natural and distilled waters, it is likely that the initial products of DNT are similar to those of TNT, and deserve additional study.

The reaction quantum yield for photolysis of DNT in air-saturated distilled water is 7.0×10^{-4} at 313 nm; since the photolyses of TNT and DNT probably occur by similar processes, the photolysis quantum yield for DNT is likely to be independent of wavelength, as is that for TNT. The quantum yield measured in air-saturated water is relevant to this environmental assessment because natural waters receiving sunlight are surface waters that are certainly air-saturated.

Using this quantum yield, we calculated the variation in the photolysis half-life for the seasons of the year according to the procedure of Zepp and Cline (1977). Table 29 summarizes the predicted half-lives at 20°, 40°, and 50° N latitudes.

Table 28

ABSORPTION SPECTRUM OF 2,4-DNT[a] IN 1% ACETONITRILE-99% WATER

Wavelength	Absorption Coefficient (M^{-1} cm^{-1})
297.5	4100
300.0	3750
302.5	3390
305.0	3030
307.5	2680
310.0	2320
312.5	1960
315.0	1784
317.5	1600
320.0	1340
323.1	1250
330.0	1070
340.0	714
350.0	535
360.0	357
370.0	268
380.0	178
390.0	89
400.0	36

[a]DNT concentration was 5.60×10^{-4} M.

Table 29

ANNUAL VARIATION OF 2,4-DNT PHOTOLYSIS HALF-LIFE*
IN SUNLIGHT IN DISTILLED WATER

Season	N Latitude 20°	40°	50°
Summer	1.8	1.8	1.9
Fall	2.3	3.6	5.4
Winter	2.8	6.0	11.5
Spring	1.9	2.2	2.5

*Half-lives are in 24-hour days (10 hours sunlight).

At 40° latitude, the 2,4-DNT sun photolysis half-life in distilled water will range from 1.8 days in midsummer to 6.0 days during midwinter. We measured a half-life of 1.8 days (43 hours) during summer, which is in excellent agreement with the calculated rate. From the natural-water sunlight photolysis experiment, however, the photolysis rates in natural waters will be faster.

3. Biotransformation

a. Screening Test

Screening tests for 2,4-DNT biotransformation were conducted with Waconda Bay water. We found that 2,4-DNT was readily biotransformed by natural water microorganisms that can use 2,4-DNT as a sole carbon source. More than 90% of 10 ppm 2,4-DNT in aerated water was transformed in 6 days with 2-3 days of lag period. The 2,4-DNT concentration in a sterile-water control did not change during 2 weeks of incubation. Also, 2,4-DNT in the presence of 30 ppm yeast extract had a similar transformation rate during 6 days of incubation. In a flask with N_2-flushed head space, more than 90% of the 2,4-DNT disappeared in 6 days. In the presence of yeast extract, 12 days were necessary to use 90% of the substrate anaerobically. It seems that some nutrients in the yeast extract may repress 2,4-DNT utilization and cause a

diauxic utilization. The yeast extract may provide nutrients for aerobic bacteria growth, which resulted in the consumption of dissolved oxygen, thus creating an anaerobic condition that slowed the activity of 2,4-DNT degrading microorganisms.

The 2,4-DNT utilizing microorganisms from Waconda Bay were inoculated into a shaker flask containing 2,4-DNT and basal-salts medium for culture enrichment. By gradually increasing 2,4-DNT concentration, the mixed culture could grow in 120 ppm 2,4-DNT.

The transformation of 2,4-DNT in 1, 3, and 10 ppm media inoculated with diluted broth of 100 ppm 2,4-DNT medium flask are shown in Figure 31. With an initial cell count of 3×10^3 cells ml^{-1}, 2,4-DNT was consumed in 2-3 days at 1-10 ppm concentration. For each µg of 2,4-DNT consumed, about 3×10^6 cells were produced under the growth conditions.

b. Transformation of Ring-Labeled ^{14}C-2,4-DNT

Since 2,4-DNT can be used as sole carbon source by microorganisms, the benzene ring was assumed to be cleaved and the carbon utilized. To confirm this, ring-labeled ^{14}C-2,4-DNT was incorporated in basal-salts medium containing 100 ppm 2,4-DNT in a sealed flask, and Waconda Bay 2,4-DNT-degrading organisms were inoculated.

The percent of residual radioactivity of the added ^{14}C in the medium and accumulated $^{14}CO_2$ activity trapped in the alkaline solution are shown in Figure 32.

Total $^{14}CO_2$ trapped accounted for 59% of the initial ^{14}C after 7 days of incubation. The residual activity in the medium accounted for 22% of the initial ^{14}C. This result showed that the 2,4-DNT ring was cleaved and that the chemical was ultimately biotransformed to CO_2. The medium was extracted with ethyl acetate at neutral and acidic pH, and the concentrated extracts were analyzed by TLC. Radioautography of the TLC plate did not show detectable major intermediates. These results are contrary to those of 2,4,6-TNT, which was not oxidized to CO_2 and whose major metabolites are reduced derivatives.

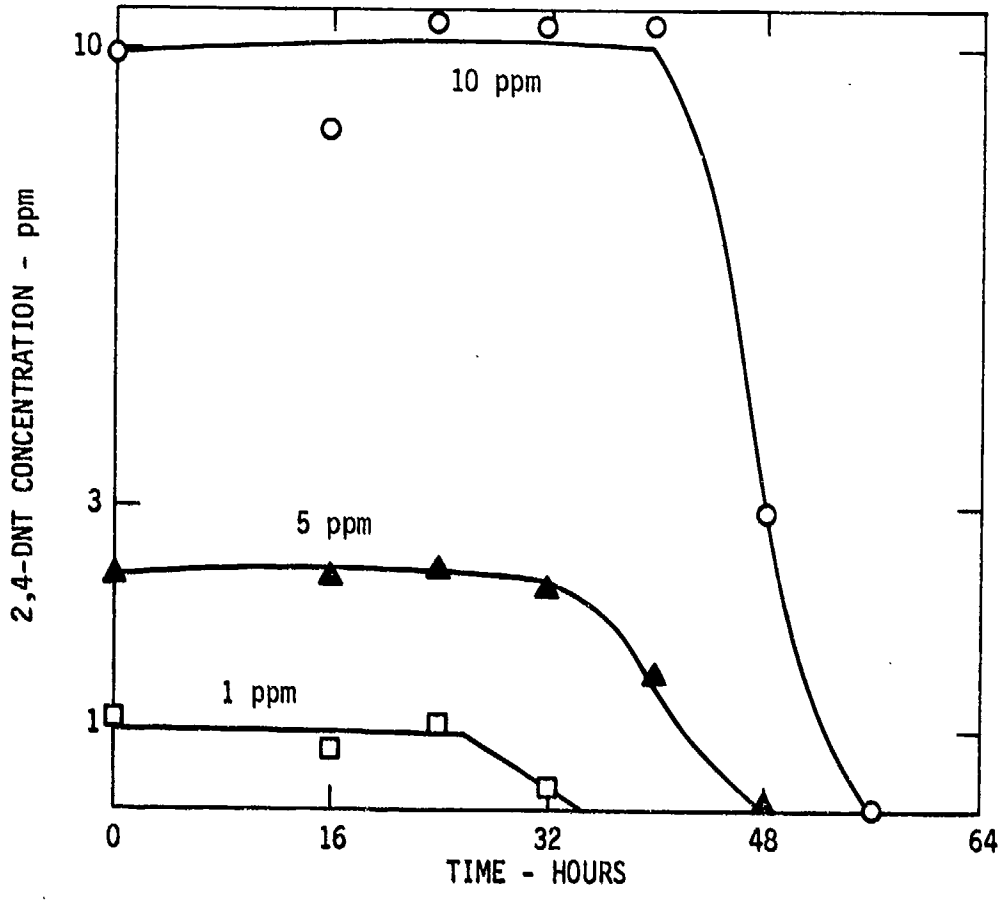


FIGURE 31 BIOTRANSFORMATION OF 2,4-DNT IN SHAKER FLASK

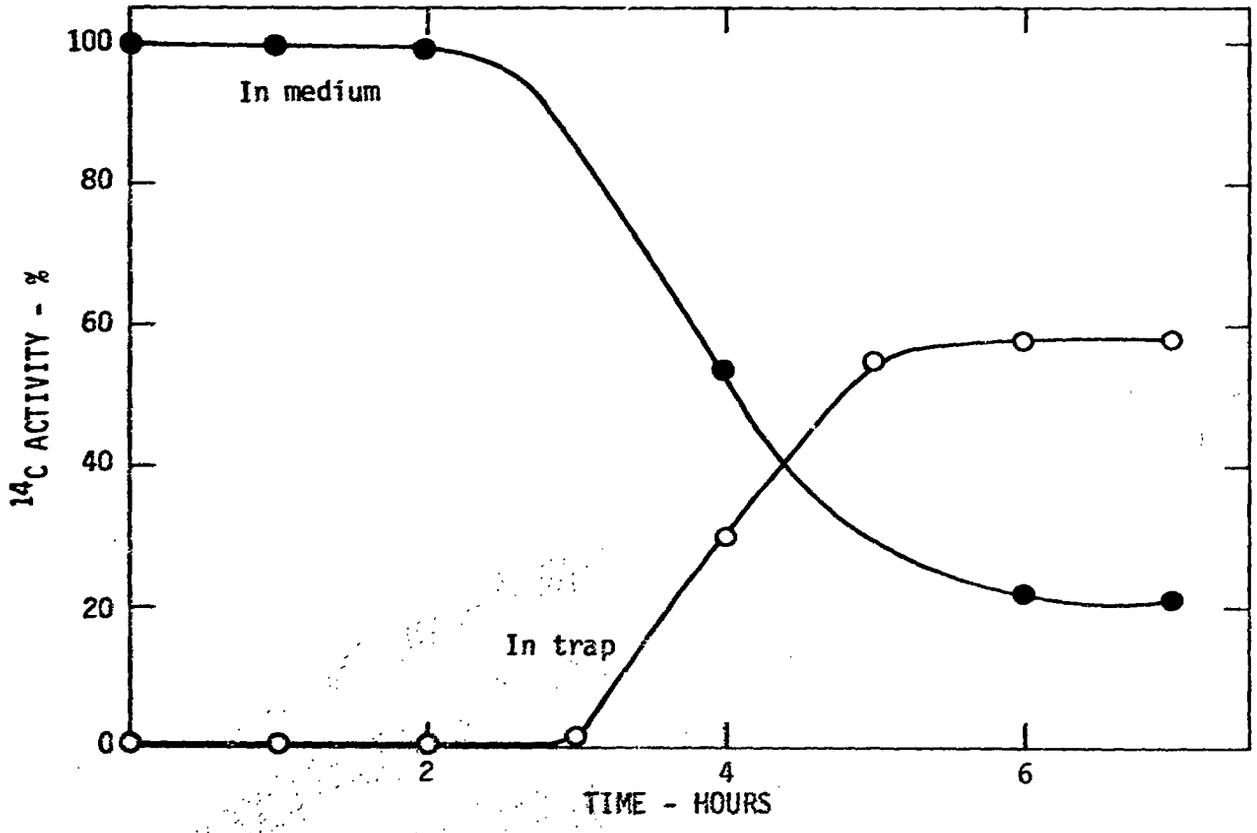


FIGURE 32 RESIDUAL ^{14}C ACTIVITY OF RING-LABELED 2,4-DNT IN MEDIUM AND IN CO_2

c. Biotransformation Rate Constant

Waconda Bay 2,4-DNT microorganisms were grown in basal-salts medium containing 100 ppm 2,4-DNT, centrifuged, washed, and used for a high-cell-population biotransformation study. Figure 33 shows the pseudo-first order transformation of 2.7 ppm 2,4-DNT with 1.9×10^7 cells ml^{-1}. There was an initially slow transformation period, followed by faster first-order transformation. The half-life of 2,4-DNT was estimated from the curve to be 0.40 h, which corresponds to $k_{b^1} = 1.7\ h^{-1}$ and $k_{b^2} = (1.7/1.9 \times 10^7) = 9.1 \times 10^{-8}$ ml $cell^{-1}\ h^{-1}$. In a second experiment with 2.5 ppm 2,4-DNT and with 5.3×10^7 and 1.07×10^8 cells ml^{-1} cell concentrations, the results are shown in Figure 34. The half-lives of 2,4-DNT at first-order transformation were 0.41 and 0.27 h for (A) and (B), respectively. The pseudo-first-order rate constants were 1.7×10^{-1} and $2.6 \times 10^{-1}\ h^{-1}$ and second-order rate constants were 3.2×10^{-8} and 2.4×10^{-8} ml $cell^{-1}\ hr^{-1}$, respectively. The average second-order rate constant from 3 transformation studies was 4.7×10^{-8} ml $cell^{-1}\ h^{-1}$.

d. Metabolites

GC and HPLC profiles of the 2,4-DNT biotransformation showed no extensive buildup of metabolic products. Attempts were made to collect fractions from the HPLC profile (Figure 35) for probe mass-spectrometry analysis, and one component was identified as 4-amino-2-nitrotoluene. Although definitive mass spectral data could not be obtained for the later-eluting components, their chromatographic behavior parallels that of the nitroazoxytoluenes observed in the TNT biotransformation study. We also observed that 2,6-dinitrotoluene, a minor impurity in the starting 2,4-DNT was not degraded by the 2,4-DNT organisms and accumulated in the medium.

e. Discussion

The compound 2,4-DNT can be readily biotransformed as a sole carbon source by natural microorganisms. The rate is fairly fast, and the chemical can be ultimately transformed to CO_2. Some fungi transformed

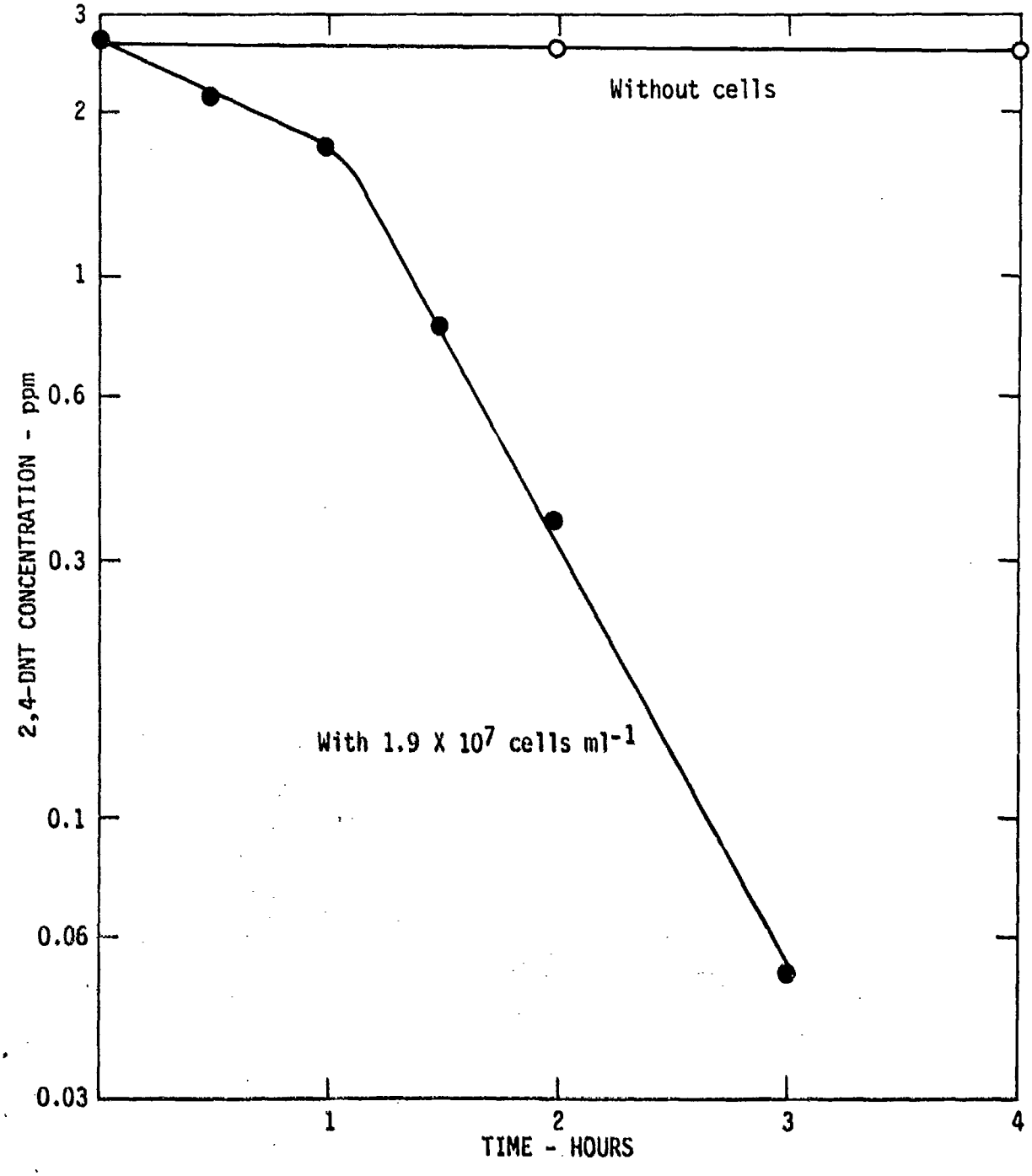


FIGURE 33 2,4-DNT BIOTRANSFORMATION WITH AND WITHOUT CELLS

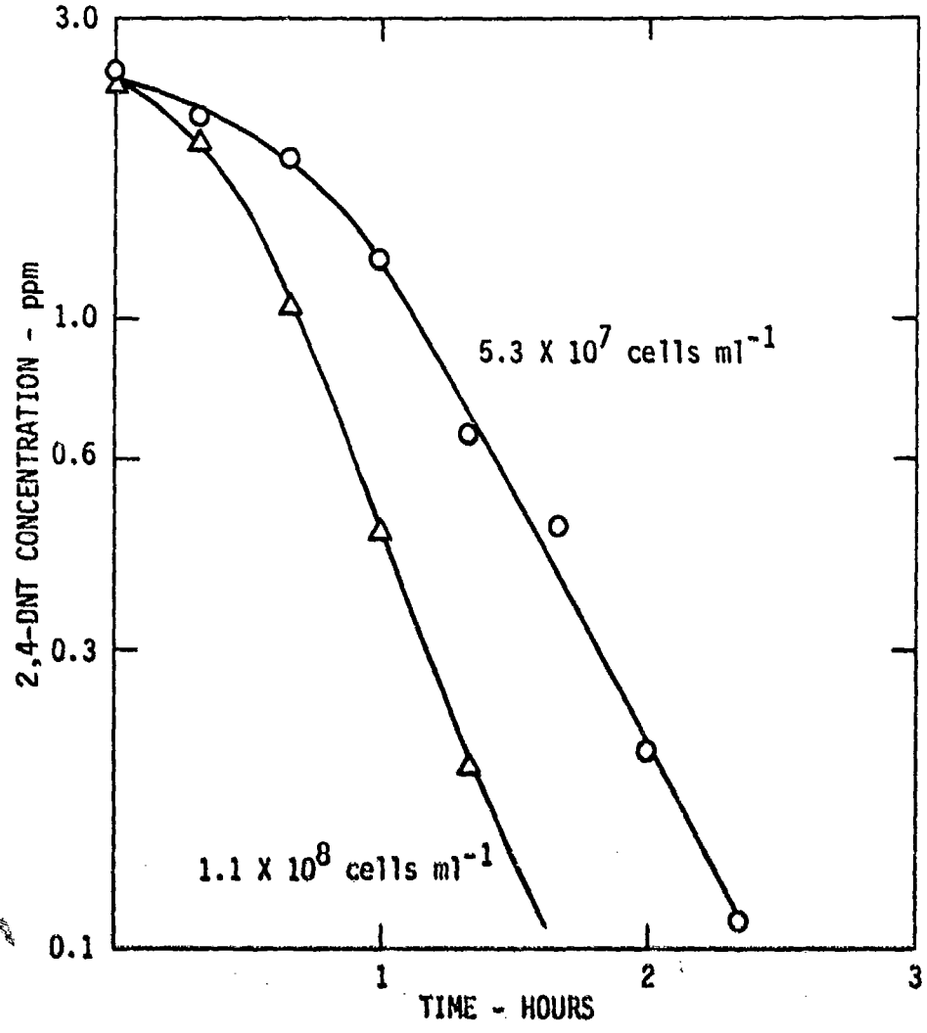


FIGURE 34 2,4-DNT BIOTRANSFORMATION WITH CELLS

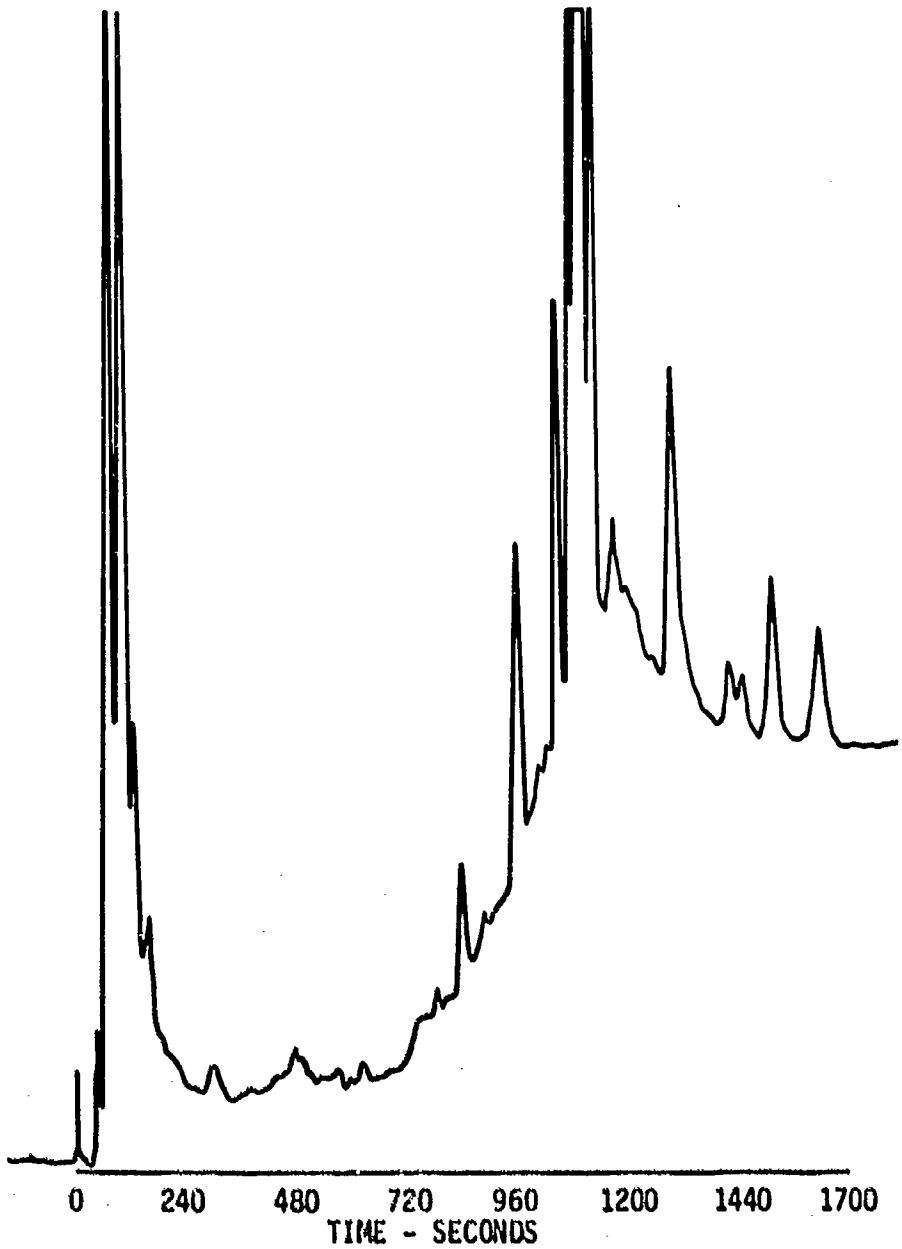


FIGURE 35 HPLC PROFILE FOR 2,4-DNT METABOLITES IN BIOTRANSFORMATION STUDY

2,4-DNT in basal-salts medium with glucose (Parrish, 1977), and reduced compounds--2-amino and 4-amino-derivatives and their azoxyderivatives--were identified. Similar compounds were identified in the broth of fungus Microsporum species when exposed to 2,4-DNT (McCormick et al., 1978). However, no environmental fate study was reported. Our finding that 2,4-DNT can be readily biodegraded to CO_2 by bacteria showed that 2,4-DNT will not be persistent in the environment.

4. Biosorption

Our preliminary study with the viable cells of 4 ATCC biosorption test organisms mixture showed that 2,4-DNT can be biotransformed by this mixture. Therefore, a biosorption study was conducted with heat-killed cells. With a dead cell mixture of 1.36 mg/ml (dry weight) and an initial 2,4-DNT concentration of 11 ppm, the average biosorption coefficient and standard deviation was 64 ± 9. This value is very low and environmentally not important.

5. Sediment Sorption

The screening isotherm was measured using 2 flasks containing sediment and 2,4-DNT solution, one flask with sediment only and 1 flask with 2,4-DNT solution only. Table 30 lists the amount of each substance added to each flask.

The sediment and glassware were autoclaved. Water and 2,4-DNT solution were filtered through a 0.22-μm filter. These precautions were taken because the biotransformation of 2,4-DNT was fast compared to the time required to equilibrate the 2,4-DNT with the sediment. Samples were taken shortly after preparation of the isotherm mixtures and after 240 hours. The samples were centrifuged and the 2,4-DNT concentrations in the supernatant and sediment were measured by HPLC. Results of these measurements are reported in Table 31.

The low sediment partition coefficient suggests that sediment sorption will not be a significant loss process for 2,4-DNT.

Table 30

MATERIALS USED FOR 2,4-DNT SORPTION EXPERIMENTS

Flask No. / Material	1	2	3	4
H_2O (ml)[a]	100	100	100	200
2,4-DNT solution (ml)	100	100	100	--
Sediment (g)	--	20	20	20
Saturated $HgCl_2$ solution (drops)	10	10	10	10

[a]Volume is total volume of H_2O plus sediment.

Table 31

DISTRIBUTION OF 2,4-DNT IN SEDIMENT SORPTION FLASKS AND CALCULATED K_p VALUES

Time (h)	Material	1	2	3	4
0	2,4-DNT in Supernatant (µg/ml)	75	25	24	0
	2,4-DNT in Sediment (µg/g)	--	4.1	3.9	0
	K_p	--	7.1	3.9	--
240	2,4-DNT in Supernatant (µg/ml)	57	22	18	0
	2,4-DNT in Sediment (µg/g)	--	5.4	5.7	0
	K_p	--	12	12	--

6. Volatilization

The rate constant for volatilization of 2,4-DNT in the aquatic environment (k_v^C) can be estimated by the method outlined in Section III, C of this report, using equation 26

$$k_v^C = \frac{H_c k_g^W B}{LRT} \quad , \tag{26}$$

where H_c,* L, R, T, and k_g^W can be estimated or measured independently. The value of B, which is a ratio of diffusion constants (see page 16) was estimated from a series of laboratory measurements of k_v^C and k_g^W which are used to solve for B in equation 27.

$$B = \frac{k_v^C LRT}{H_c k_g^W} \quad . \tag{27}$$

Eight experiments were performed to evaluate simultaneously k_v^C for 2,4-DNT and k_g^W for water. The results are summarized in Table 32.

The average value of B (0.98) was then used to estimate a value of k_{ve}^C for an aquatic water body by assuming that k_g^W = 2100 cm h^{-1} (Smith and Bomberger, 1980), L = 200 cm depth, and T = 293 K.

$$k_v^C = \frac{0.12 \times 0.98 \times 2100}{200 \times 62.3 \times 293}$$

$$= 6.6 \times 10^{-5} \ h^{-1} \quad .$$

This rate constant is much smaller than the rate constants for photolysis and biotransformation of 2,4-DNT. Therefore we believe that volatilization will not be an important competing process in the aquatic environment.

*We estimated a value of H_c of 0.12 torr M^{-1} from the vapor pressure of 1.1×10^{-4} (20°C) extrapolated from the data of Pella (1977) and from our measured value of the solubility of 9.5×10^{-4} (Smith et al., 1980).

Table 32

VOLATILIZATION RATE DATA FOR 2,4-DNT
(H_C = 0.116 torr M^{-1})

Run No.	k_v^C(hr^{-1})	k_g^W cm hr^{-1}	L (cm)	T (°K)	B
1	5.25 ± 10^{-3}	4800	7.5	290	1.30
2	2.75 ± 10^{-3}	3300	7.5	290	0.99
3	2.63 ± 10^{-3}	3500	7.6	291	0.88
4	2.60 ± 10^{-3}	3900	7.8	292	0.82
5	4.51 ± 10^{-3}	6400	8.3	292	0.92
6	3.40 ± 10^{-3}	4700	8.3	292	0.94
7	2.65 ± 10^{-3}	3400	8.3	293	0.85
8	3.68 ± 10^{-3}	4100	8.3	294	1.20

Average value of B = 0.98 ± 0.14[a]

[a]95% confidence limit.

7. Model Simulation Results

a. New River

In the model simulations of the New River, a first order biotransformation rate constant of 1.20 day^{-1} at 10^6 cells ml^{-1} was applied for both mean- and low-flow cases; a first-order photolysis rate constant of 3.0 day^{-1} was used in the low-flow case, and one of 2.0 day^{-1} in the mean-flow case. A sorption partition coefficient of 14 was used in this analysis. Figure 36 shows the simulated 2,4-DNT concentrations for both cases. The expected concentration of 2,4-DNT is higher in the low-flow case than in the mean-flow case because of dilution. However, the 2,4-DNT concentration will decrease at a slightly faster rate during low flow. During cloudy days, the 2,4-DNT concentrations may be 50% higher than the projected values, resulting in concentrations of 60 ppb (low flow) and 11 ppb at the end of the studied river reach.

b. Waconda Bay

Rate constants used in the Waconda Bay simulation are presented in Table 33. Although the partition coefficient and biotransformation rate constant are the same as those for the New River, the photolysis rate constant varies with the depth and sediment loading in the water body.

Projections of 2,4-DNT concentrations in Waconda Bay are presented in Figure 37 for discharge rates of 220 liters s^{-1} and 1100 liters s^{-1}. It is estimated that 2,4-DNT concentrations as high as 10 ppb may be observed in Harrison Bay (see Figure 23) and possibly up to 1 ppb in the Tennessee River.

8. Conclusions

The environmental fate of 2,4-DNT will be controlled primarily by photolysis and biotransformation. Microorganisms readily metabolize 2,4-DNT, to CO_2 as the final product. Therefore, 2,4-DNT is not expected to persist in the environment. High loading conditions during cloudy weather in winter months would cause 2,4-DNT to travel the farthest from the discharge point.

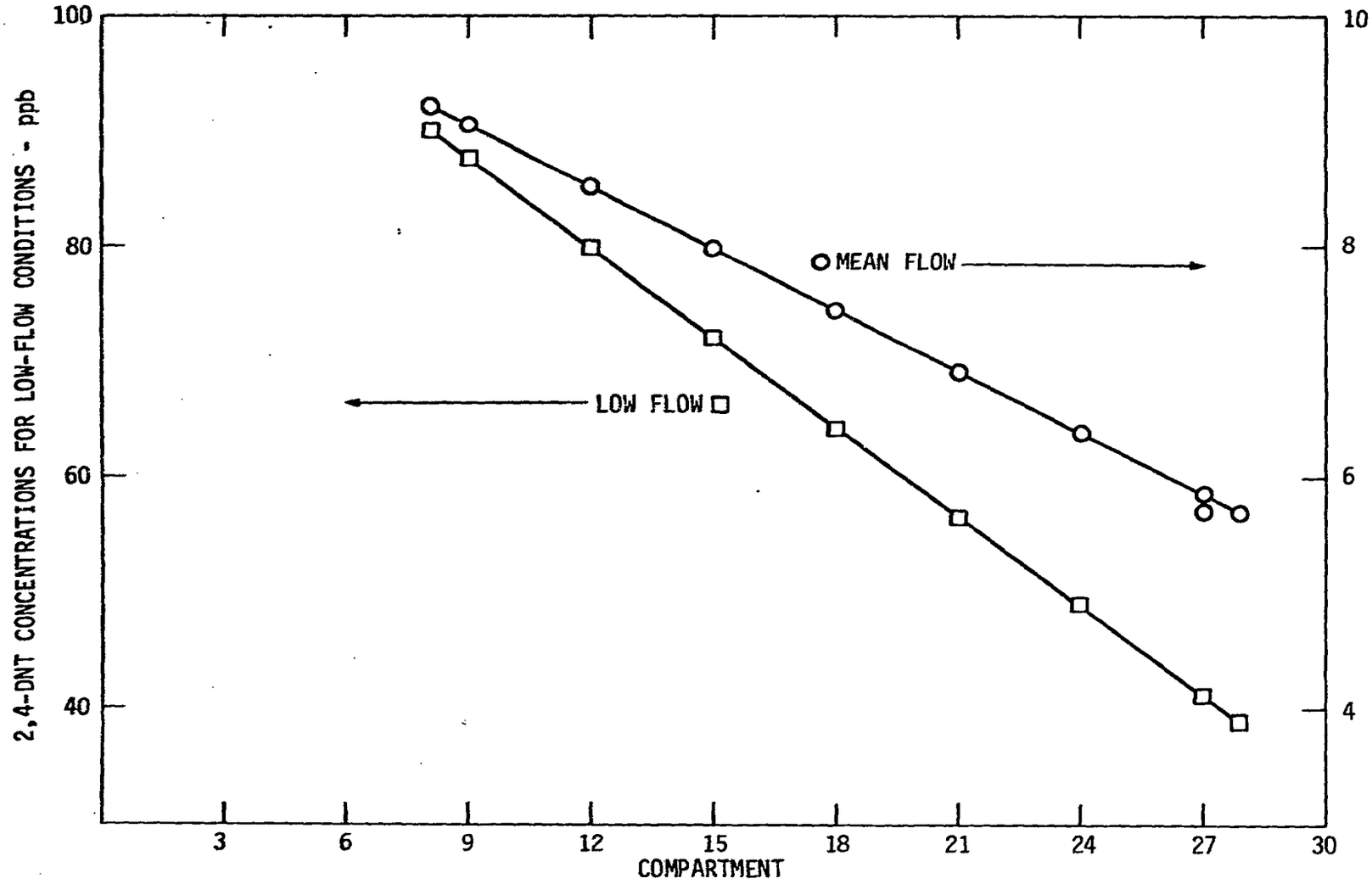


FIGURE 36 SIMULATED 2,4-DNT CONCENTRATIONS IN THE NEW RIVER

Table 33

ESTIMATED RATE CONSTANTS OF 2,4-DNT TRANSFORMATION AND TRANSPORT USED IN MODEL SIMULATION FOR WACONDA BAY

Compartment	Partition Coefficient	Photolysis (Day^{-1})	Biodegradation (Day^{-1})
1	14	3.0	1.2
2	14	3.0	1.2
3	14	3.0	1.2
4	14	2.5	1.2
5	14	2.0	1.2
6	14	1.5	1.2
7	14	1.5	1.2
8	14	1.5	1.2
9	14	1.5	1.2
10	14	1.5	1.2
11	14	1.5	1.2
12	14	1.5	1.2

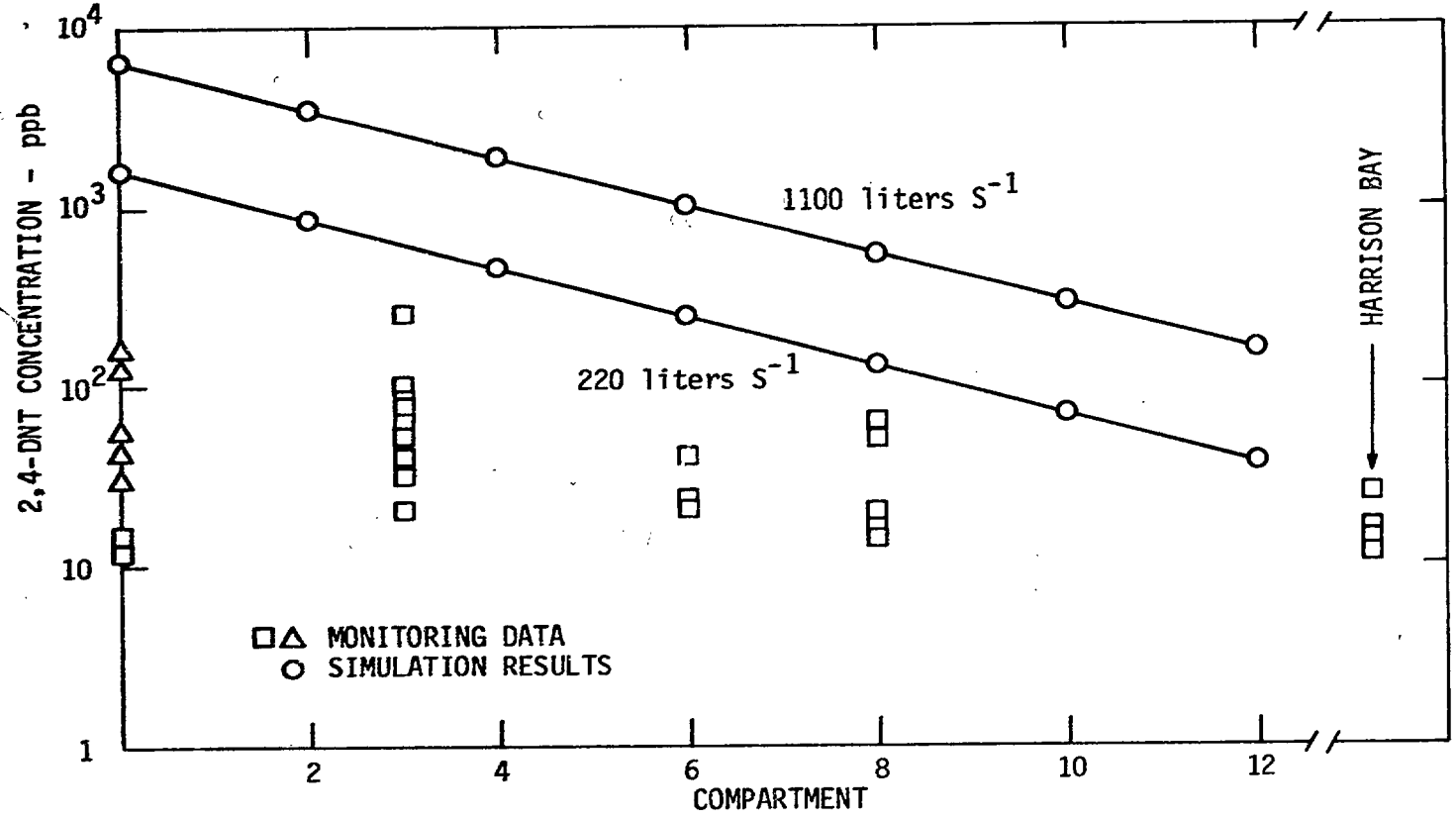


FIGURE 37 SIMULATED 2,4-DNT CONCENTRATIONS IN WACONDA BAY

D. Trinitroglycerin (TNG)

1. Background

The Phase I literature review indicated that TNG might be subject to photochemical transformation (Urbanski, 1964) and that therefore this fate process should be investigated in the aquatic environment. Hydrolysis of TNG was extensively studied by Rosseel et al., (1974). From his data, TNG hydrolysis was considered to be slow, and would contribute little to the loss of TNG in the environment.

Biotransformation has not been thoroughly investigated and this fate process was considered to be important because TNG is metabolized rapidly in mammalian systems (DiCarlo, 1975). Sorption processes were estimated not to be important, but partition coefficients were obtained because none was available from the literature.

2. Photochemistry

The UV absorption spectrum of TNG (Table 34) showed only weak absorption above the solar spectrum cutoff of about 290 nm, and therefore, the direct photolysis of TNG was expected to be slow. Use of the data in Table 32 to calculate the direct photolysis half-life of TNG assuming a maximum possible quantum yield of unity (1.0) indicated that the half-life of TNG in pure water will be at least 5 days in summer.

To verify the expected slow direct photolysis of TNG and to determine whether indirect photolysis of TNG in natural waters was important, solutions of 5.8 ppm (2.6×10^{-5} M) TNG were prepared in distilled water and in filter-sterilized samples of two natural waters and exposed to sunlight from 19 December 1979 to 23 January 1980 in Menlo Park. The New River water sample was taken downstream from a TNG-manufacturing facility. The Searsville Pond water was from a naturally eutrophic wetlands area in Menlo Park and uncontaminated with munitions chemicals; the Searsville Pond water was used because the photolysis rate constant of other munitions chemicals (TNT and DNT) have been found to be

Table 34

ABSORPTION SPECTRUM OF TNG[a]

Wavelength (nm)	Absorption Coefficient ($M^{-1}cm^{-1}$)
297.5	6
300.0	5
302.5	5
305.0	4
307.5	3
310.0	2
312.5	2
315.5	1
317.5	1
320	1
323.1	1
330.0	0
340.0	0
350	0
360	0

[a] [TNG] = 3×10^{-3} M in 100% H_2O, 10-cm cell. Because photolysis of TNG is not an important fate, more precise data were not obtained.

accelerated by transformation products. The rate constants measured in these experiments are given in Table 35. The dark controls for

Table 35

SUNLIGHT PHOTOLYSIS OF 5.8 PPM TNG[a]

Water	Rate Constant $k_p \times 10^7$ (s^{-1})[b]	Half-life (days)[c]
Distilled	0.69 ± 0.07	116
New River[d]	1.4 ± 0.09	57
Searsville Pond[d]	1.1 ± 0.1	73

[a] 5.8 ppm = 2.56×10^{-5} M TNG.

[b] Rate constants corrected for unknown loss process in dark control.

[c] Half-lives are in 24-hour days (10 hours sunlight).

[d] Natural waters were filtered through a 0.45-μ filter.

these experiments were kept at 20°C for the 34 days, when analysis found TNG losses of approximately 7% and 12% in the New River and Searsville Pond waters, respectively; no TNG loss was found in the distilled water solutions. The data in Table 35 are corrected for the unknown loss process occurring in the dark controls.

The data in Table 35 show that TNG slowly photolyzes in distilled and natural waters, with unknown process(es) contributing to a photolysis rate in the natural waters approximately twice that in distilled water. Further studies of the photolysis of TNG were not conducted because of the facile biotransformation of TNG.

3. Biotransformation

a. Screening Test

Biotransformation screening tests for TNG were conducted with water obtained from New River near a TNG waste outflow at Radford AAP, Radford, Virginia. TNG (10 ppm) in New River water was biotransformed within 13 days either when added alone, with river sediment, or with 50 ppm yeast extract under both aerobic and microaerophilic (headspace flushed with N_2) conditions. When the TNG-acclimated microorganisms from the aerobic bottle were inoculated into the shaker flasks containing 10 ppm TNG in basal-salts medium, the TNG was biotransformed. These organisms are capable of utilizing TNG as a sole carbon source. When the TNG concentration in the media was increased, the enriched culture could grow in 120 ppm TNG and basal-salts medium. However, the cells tended to aggregate in the shaker flask instead of forming a homogeneous cell suspension, so the normal means of growth observation by cloudiness became difficult and plate counts became inaccurate. The cell flocculation phenomenon was reduced by using pure water (passed through a Milli Q filter) and growing the cells in flasks agitated with magnetic stir bars instead of in shaker flasks. The cell plate count was improved as follows. One ml of medium broth was added to a Waring blender with 99 ml of water, the mixture was blended for 1 minute, and then serial dilutions were made for plating on agar medium. Figure 38 shows the TNG degradation profile in a jar fermenter and the microbial growth as a function of time. Cell yield was 1×10^6 cells per µg of TNG consumed. In a flask containing 120 ppm TNG in medium, analysis of nitrite and nitrate by HPLC and a 210-nm UV detector using an ion-exchange column showed that 96% of the theoretical amount of nitrogen was liberated as nitrite; only trace amounts of nitrate were produced after 5 days of incubation.

b. Biotransformation Rate Constant

TNG-transforming microorganisms were grown in a jar fermenter and cells were harvested after about 4 days of incubation, when 95% of the TNG had been consumed. The cells were centrifuged, washed, and

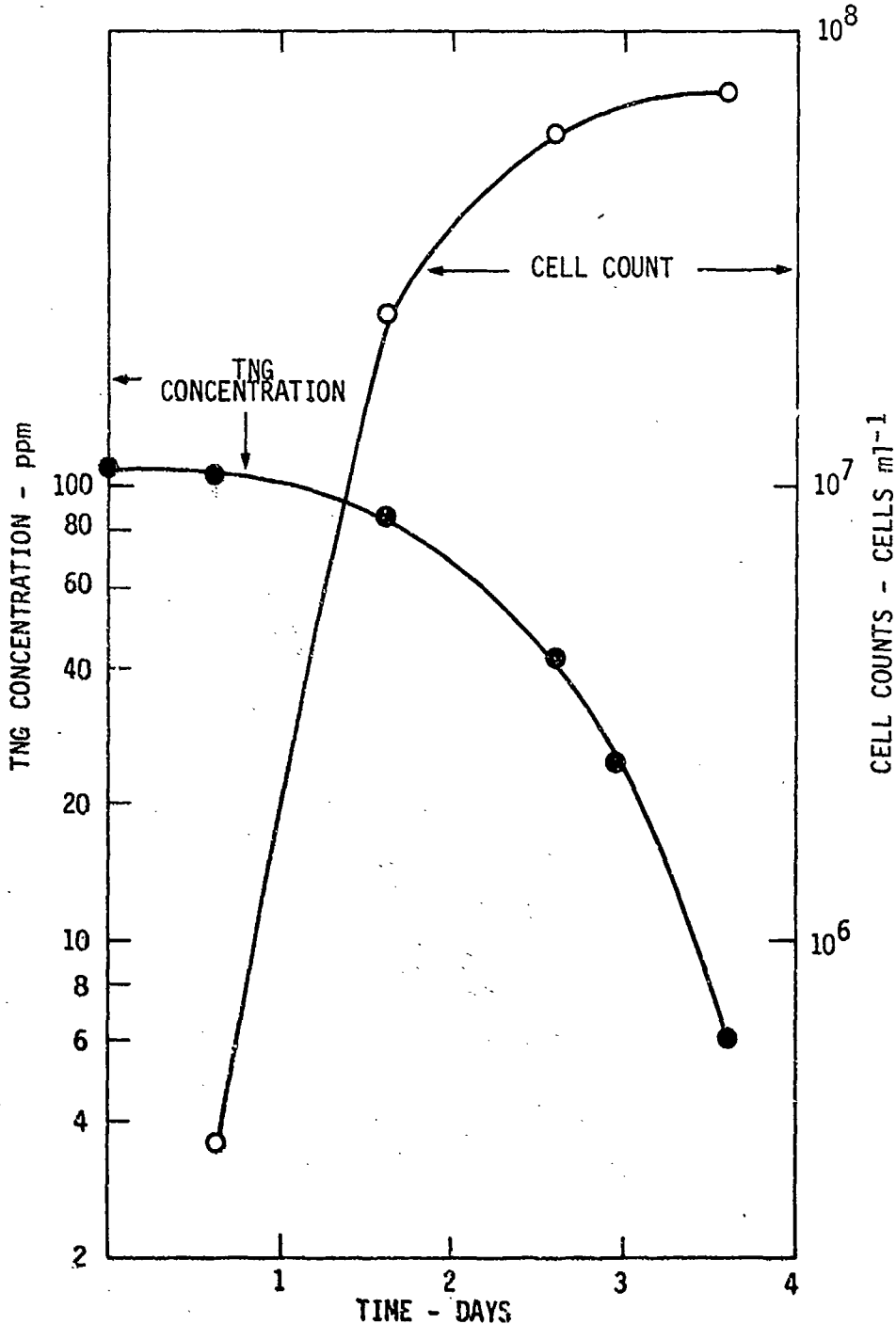


FIGURE 38 BIOTRANSFORMATION OF TNG IN JAR FERMENTERS

resuspended in a small volume of basal-salts medium. A high-cell-population transformation study was conducted using an initial TNG concentration of 2.5 ppm and a cell concentration of 1.27×10^9 cells ml^{-1}. The result is shown in Figure 39. TNG degraded with a pseudo-first-order rate constant (k_{b_1}) of 0.60 h^{-1}. The calculated second-order rate constant was 4.7×10^{-10} ml $cell^{-1}$ h^{-1}.

The transformation rate constant was also studied in an experiment measuring transformation during the growth phase. TNG-transforming organisms were grown in a flask containing 50 ppm TNG in basal-salts medium. After more than 90% of the TNG was transformed, 5 ppm TNG was added and the stirred medium was analyzed every 2 hours for 8 hours. Figure 40A shows the results of an experiment in which TNG was added after 4 days of growth. Because of the residual TNG from the original medium, the total TNG at the start of the experiment was 9.7 ppm. The cell count remained almost constant over the 8-h period at 8.1×10^7 cells ml^{-1}. The pseudo-first-order rate constant from the curve was determined to be 8.6×10^{-2} h^{-1}, and the calculated second-order rate constant was 1.06×10^{-9} ml $cell^{-1}$ h^{-1}. In a second experiment, 5 ppm TNG was added to a 5-day-old flask to give an initial concentration of 10.2 ppm. The average cell concentration was 5.3×10^7 cell ml^{-1} over 8 h. The pseudo-first-order rate constant was 7.6×10^{-2} h^{-1} and the second-order rate constant was 1.43×10^{-9} ml $cell^{-1}$ h^{-1} (Figure 40B). The average second-order rate constant of the two flasks was 1.25×10^{-9} ml $cell^{-1}$ h.

c. Metabolites

The broth from TNG biotransformation experiments was analyzed directly and as the ethyl acetate extract by HPLC. No evidence of dinitro- or mononitroglycerols was found by UV detection or by the methods reported by Spanggord and Keck (1980) using the TEA detector. These metabolites, if formed, may be transformed more rapidly than TNG and therefore do not accumulate in the medium. Nitrite was found to be the major product resulting from the nitro groups.

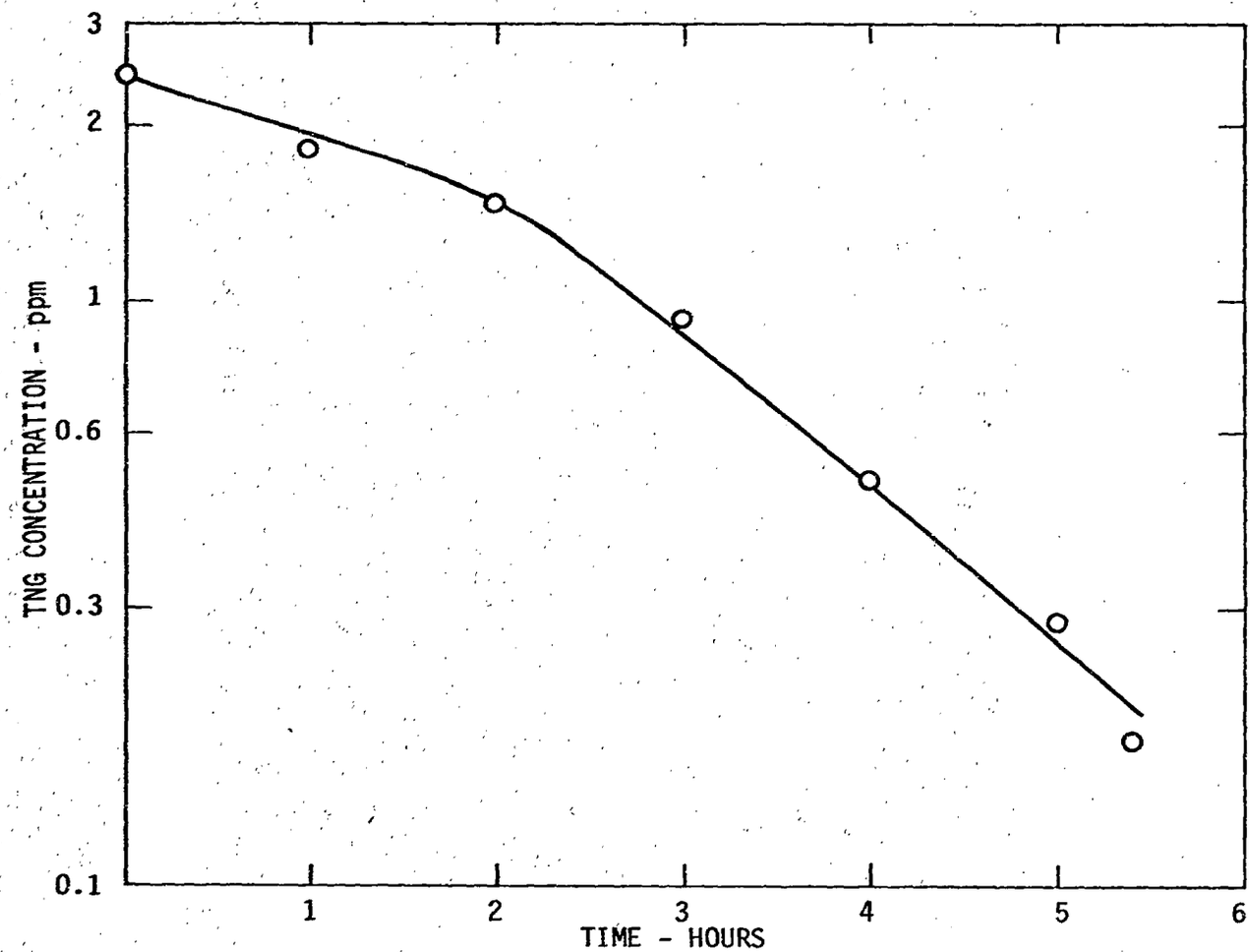


FIGURE 39 TNG BIOTRANSFORMATIONS WITH HIGH CELL POPULATIONS

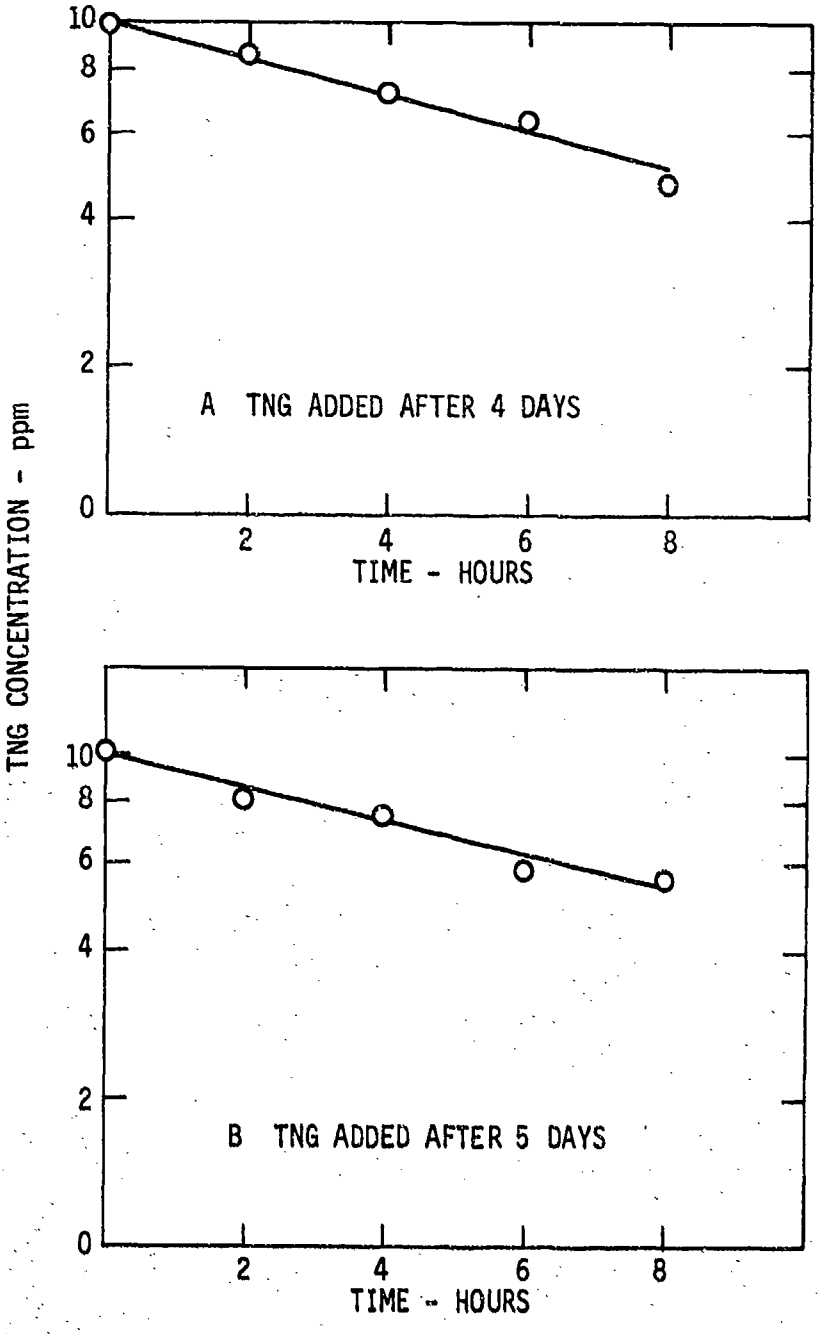


FIGURE 40 TNG BIOTRANSFORMATION DURING GROWTH PHASE OF MICROORGANISMS

d. Discussion

Although Wendt et al. (1978) reported that TNG was biodegraded by microorganisms in the presence of glucose, they found that TNG was not a suitable carbon or nitrogen source for growth. Our results are the first indication of microbial degradation utilizing TNG as a sole carbon source. Wendt et al. (1978) found that dinitro- and mononitroglycerols were produced in TNG-glucose medium. These metabolites were not found in our study, indicating that the first nitro-group removal may be the rate-limiting step under our growth conditions. Because the microorganisms were able to utilize TNG as the sole carbon source for growth, we expect that TNG will eventually be mineralized in the environment.

4. Biosorption

A TNG-biosorption study was conducted with heat-killed cells of 4 ATCC test organisms to avoid biotransformation of the compound. With 5.6 mg ml^{-1} cells and 25 ppm TNG, the average biosorption coefficient and standard deviation was 37 ± 10. This number is very low and is not environmentally significant.

5. Sediment Sorption

The screening isotherm for TNG was measured by preparing flasks containing TNG and sediment, 1 flask containing only TNG, and 1 flask containing only sediment. The sediment used was a sterilized sample from the Holston River (upstream). To suppress biologic activity, $HgCl_2$ was added to each flask. The specific amounts of the substances added to each flask are listed in Table 36.

Table 36

MATERIALS USED FOR TNG SORPTION EXPERIMENTS

Material	Flask 1	2	3	4
H_2O (ml)	115	100	100	100
TNG solution (ml)	100	100	100	--
Sediment (g)	--	20	20	20
Saturated $HgCl_2$ solution (drops)	10	10	10	10

Samples were taken at the beginning of the experiment and after approximately 1 week. The samples were centrifuged, and the supernatant and sediment were separated. The supernatant was analyzed directly by HPLC, and the sediment was subjected to extraction prior to HPLC analysis. The extracting solvent was ethyl acetate or acetonitrile. No significant difference was observed between ethyl acetate and acetonitrile extracts in the results of the isotherm. The results of these isotherm measurements are summarized in Table 37. The K_p and K_{oc} values are low, suggesting that sediment sorption will not be a significant loss process for TNG in the aquatic environment.

6. Model Simulations

a. Estimated Rate Constants--New River

Biotransformation experiments conducted with a high TNG concentration and a high cell population indicated that the calculated second-order rate constant ranged from 1.4×10^{-9} to 4.7×10^{-10} ml $cell^{-1}$ h^{-1}. With a cell concentration of 10^9 cells ml^{-1}, the biotransformation half-life of TNG was estimated to be 1 hour. The biotransformation half-life in natural waters (10^6 cells ml^{-1}) was calculated to be 30 days. If the cell count in the New River is assumed to be 10^6 cells ml^{-1}, the biodegradation rate constant will be 1.0×10^{-3} h^{-1}; this value was used for the model simulation. Photolysis of TNG was observed to be slow;

Table 37

DISTRIBUTION OF TNG IN SORPTION STUDIES AND THE CALCULATED K_p and K_{oc} VALUES

Time (h)	Material	Flask No. 1	2	2A[a]	3	3A[a]	4
0	TNG in supernatant ($\mu g\ ml^{-1}$)	15.8	12.2		12.5		0
	TNG in sediment ($\mu g\ g^{-1}$)	--	Lost		38.5		--
	K_p	--	--		3.08		--
	K_{oc}	--	--		93		--
187	TNG in supernatant ($\mu g\ ml^{-1}$)	13.7	4.09	6.87	8.53	7.06	0
	TNG in sediment ($\mu g\ g^{-1}$	--	109	Lost	122	126	--
	K_p	--	27	--	14	18	--
	K_{oc}	--	810	--	430	540	--

[a]Acetonitrile used as extracting solvent. Ethyl acetate used as solvent with other flasks.

the screening test for photolysis of TNG in the New River water indicated that the half-life was about 60 days. The partition coefficient, K_p, was determined to be 26.7 and 14.3. These partition coefficients are low and indicate that little TNG will be absorbed on the suspended sediment. Therefore, the partition coefficient of 20 was used for model simulations.

b. Simulation Results--New River

Because the photolysis and biotransformation rate constants are low, the fate of TNG in the New River appears to depend on dilution of wastestreams from the New River and subsequent downstream dilutions. During low-flow periods, a dilution factor of 2×10^4 was estimated. Assuming that 33 lbs (15 kg) of TNG are discharged into the New River at a rate of 23 liters s^{-1}, the projected TNG concentration is 10 ppb. The simulation results indicate that only a small amount of TNG will be transformed through biotransformation and photolysis and that the TNG concentration will remain constant for a long distance from the discharge point until major tributaries contribute to further dilution. Under mean flow conditions, the dilution factor is estimated to be 1×10^5, resulting in concentrations of 1 ppb TNG in the New River.

7. Conclusions

TNG is expected to persist in the environment. Photolysis and biotransformation will occur slowly in natural waters, and biotransformation will be significant only when microbial populations approach 1×10^9 cells ml^{-1}. Dilution will be the primary factor for reducing TNG concentrations in the receiving water bodies.

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APPENDIX A

EXPERIMENTAL METHODS

The following sections describe the experimental methods used to study the photochemical and microbial transformation processes and the sorption and volatilization transport processes. Also described are the analytical methods used for each fate assessment.

A. Photochemical Studies

1. Laboratory Photolyses

Reaction mixtures of the chemical (4 ml) were placed in 10-mm-OD borosilicate tubes (Pyrex 7740) and photolyzed on a merry-go-round reactor (Ace Glass). The irradiation source was a Hanovia 450-watt, medium-pressure Hg lamp in a borosilicate immersion well. The distance between the irradiation source and the tubes was about 10 cm. The reaction temperature was at the ambient operating temperature of the system (~ 28°C).

The photolyses were carried out using several different filter systems, which were placed between the Hg lamp and reaction mixtures. In all laboratory photolyses, the borosilicate glass immersion well (8-mm total glass path length) served as a filter to screen out all light below 290 nm. Other filter systems used in the photolyses are described below.

- Filter system for 313 nm: Corning CS 7-54 glass filter with a 0.001 M potassium chromate solution in 3% aqueous sodium carbonate circulated in the immersion well. This system transmitted primarily the 313.2- and 312.6-nm Hg lines, which represented more than 95% of the light incident on the reaction solutions (a small Hg line at 302.2 nm was also present).
- Filter system for 366 nm: Corning Glass CS 0-52 and CS 7-60 glass filters. This pair of glass filters transmitted only the 365.1, 365.6, and 366.4 nm lines of the Hg lamp, with no other lines observed (less than 1% of the light outside the 366-nm band).

- Filter system for light above 420 nm: The filter for these photolysis experiments was a Corning 3-71 cut-off filter. The light source was a 750-watt tungsten lamp (Westinghouse). A water-cooled, 1.5-in (38-mm) thick Pyrex filter was placed between the lamp and the filter to remove infrared irradiation and thereby prevent overheating and cracking of the filter.

Sample tubes were removed from the merry-go-round reactor at appropriate times and analyzed immediately.

2. Sunlight Photolyses

Outdoor photolyses using sunlight were carried out with solutions of each chemical in pure water to verify the computer calculation of half-life in sunlight based on measured values of ϵ_λ and ϕ. Photolyzed solutions were placed in a location free of excessive reflections from walls and windows and without morning and afternoon shadows. We used 11-mm-OD borosilicate tubes held in a rack at a 60° angle to the horizon; the tubes are made from the same glass stock used in the laboratory photolyses.

3. TNT Analysis

HPLC. Aqueous TNT samples were analyzed by direct injection onto the HPLC column.

Instrument: Waters Associates Model 6000A.
Column: μ-Bondapak C_{18}, 30 cm × 4 mm.
Solvent: Kinetic experiments, 45% acetonitrile-water isocratic; product analysis, 10 to 50% acetonitrile-water, 20-min linear program.
Flow rate: 2 ml min^{-1}.
Detector: UV @ 254 nm.

Isolation of TNT initial photolysis product (PC) from TNT. A solution of 100 ppm TNT in 5% acetonitrile-water was photolyzed in a 2-cm-ID pyrex tube for about 90 min in sunlight. The photolyzed solution was washed five times with methylene chloride (B & J UV grade). The

resulting aqueous solution contained ∿ 30% TNT, 70% PC and some minor products. Approximately 30% by volume acetonitrile (B & J UV grade) was added to the aqueous solution. The PC and TNT were extracted from this solution with diethyl ether (Mallinckrodt nanograde, distilled). The ether solution was concentrated by passing a stream of nitrogen (dried over molecular sieves) over the solution.

Thin-layer chromatography (TLC). Normal-phase TLC was used to identify previously reported TNT photolysis products by comparing R_f values and colors with reference compounds.

Support: Eastman silica gel sheets, F-254
Solvent: Benzene or benzene:ether:ethanol (5:3:2)
Detection: DMSO:EDA (10:1) spray.

Reverse-phase TLC was used to elute PC to optimize the separation of PC from TNT and other minor products.

Support: Whatman KC_{18} plates
Solvent: 0.5 M NaCl-H_2O:acetonitrile at a 3:4 ratio.

Nuclear Magnetic Resonance (NMR). Proton-NMR spectra were measured with a Varian XL-100-15 spectrometer modified for multinuclear operation and equipped with a Varian Disc Fourier Transform package. A V4412 probe and 4-mm tubes were used. The probe temperature was kept at about 10°C.

4. RDX Analysis

The photolysis of RDX was followed by HPLC using direct aqueous injection under the following conditions:

Instrument: Spectra-Physics Model 3500B
Column: C_{18}-Radial Compression Column (Waters Assoc.)
Flow rate: 2 ml min^{-1}
Detector: UV at 254 nm.
Retention time: RDX, 200 s
DNT, 620 s.

Quantitation was achieved using 3,5-dinitrotoluene as an internal standard, and peak areas were integrated with a Spectra-Physics minigrator digital integrator.

Formaldehyde was determined by gas chromatography as the 2,4-dinitrophenylhydrazone derivative according to the method of Kallio et al. (1972). Nitrate and nitrite were analyzed by HPLC according to the method of Thayer (1979).

5. 2,4-DNT Analysis

The phototransformation of 2,4-DNT was monitored by HPLC under the following conditions:

Instrument: Waters Assoc. Model 6000A
Column: C_{18}-μ Bondapak column
Solvent: Acetonitrile:water (50:50)
Detector: UV at 254 nm.

Quantitation was achieved by the external standard method. Peak areas were integrated with a Spectra-Physics minigrator digital integrator.

6. TNG Analysis

The phototransformation of TNG was monitored by HPLC under the following conditions using direct aqueous injection:

Instrument: Waters Assoc. Model 6000A
Column: C_{18}-μ Bondapak column, 300 mm x 4 mm
Solvent: Acetonitrile:water (35:65)
Flow rate: 2 ml min^{-1}
Detector: UV at 254 nm.

Peak areas were integrated with a Spectra-Physics Minigrator digital integrator, and quantitation was achieved by the external standard method.

B. Biotransformation Studies

1. Water and Sediment Samples (for all tasks)

Water and sediment samples from Volunteer AAP were collected during July and August 1979 at Waconda Bay, near Chattanooga, Tennessee, where the bay receives the waste output from Volunteer AAP. TNT was not being manufactured at the time of the sample collection. Noncontaminated upstream sediment samples were collected from Harrison Bay, (Figure A-1). Samples from Holston AAP were collected at the same time at the Holston River, near Kingsport, Tennessee, where the river receives the waste output from Holston AAP production lines 1 to 5 (Figure A-2). The first sediments collected during July were lost in the airline luggage transfer and had to be replaced in August. Samples from Radford AAP, Radford, Virginia were collected from the New River, where the waste output from the TNG-nitrocellulose operations intersect the New River (Figure A-3).

Water samples for the biotransformation studies were collected after the bottom sediment was stirred so that some sediment microorganisms were included. The samples were placed in sterile glass bottles and shipped by air to SRI, where they were stored below 10°C within 48 hours. After arrival, the sediment was passed through 2-mm screen sieves and stored under refrigeration until used.

2. Screening Test and Development of Acclimated Culture

Water samples containing sediment (about 2-3% volume) were placed in a 20-liter sterile reservoir bottle and mixed well, and the sediment was allowed to settle by gravity. The supernatants were siphoned and filtered through fine-mesh polyester cloth to remove insects and unsettled particles. Biodegradation screening tests were conducted in aerated bottles. Two liters of the water samples were added to a sterile 4-liter glass bottle containing 20 ml of a solution with 2 g of phosphate buffer (pH 7.5, final concentration of buffer, 1 g liter^{-1}). The chemical under investigation, dissolved in dimethylsulfoxide (DMSO)

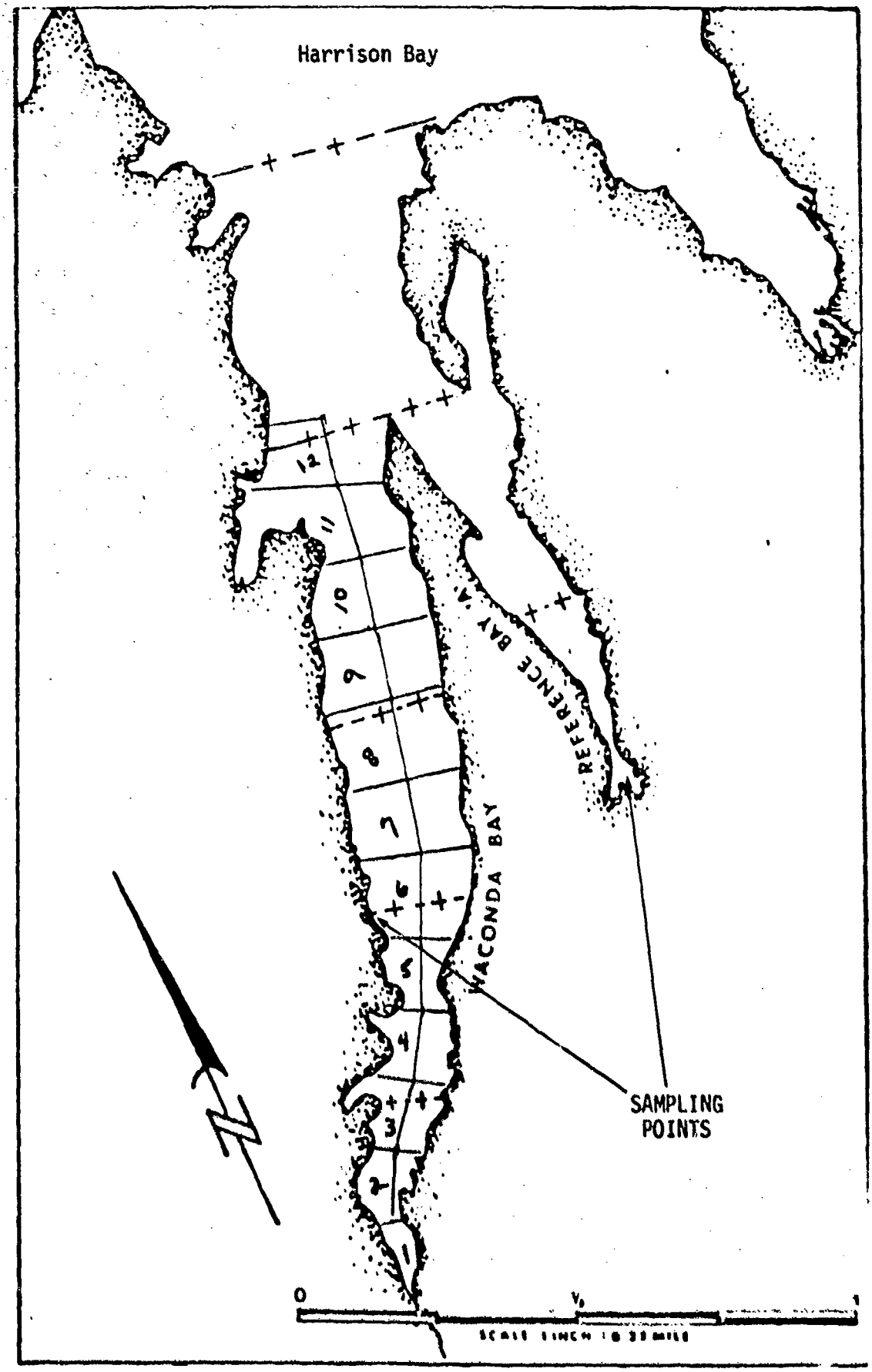


FIGURE A-1 SAMPLING POINTS, VOLUNTEER AAP

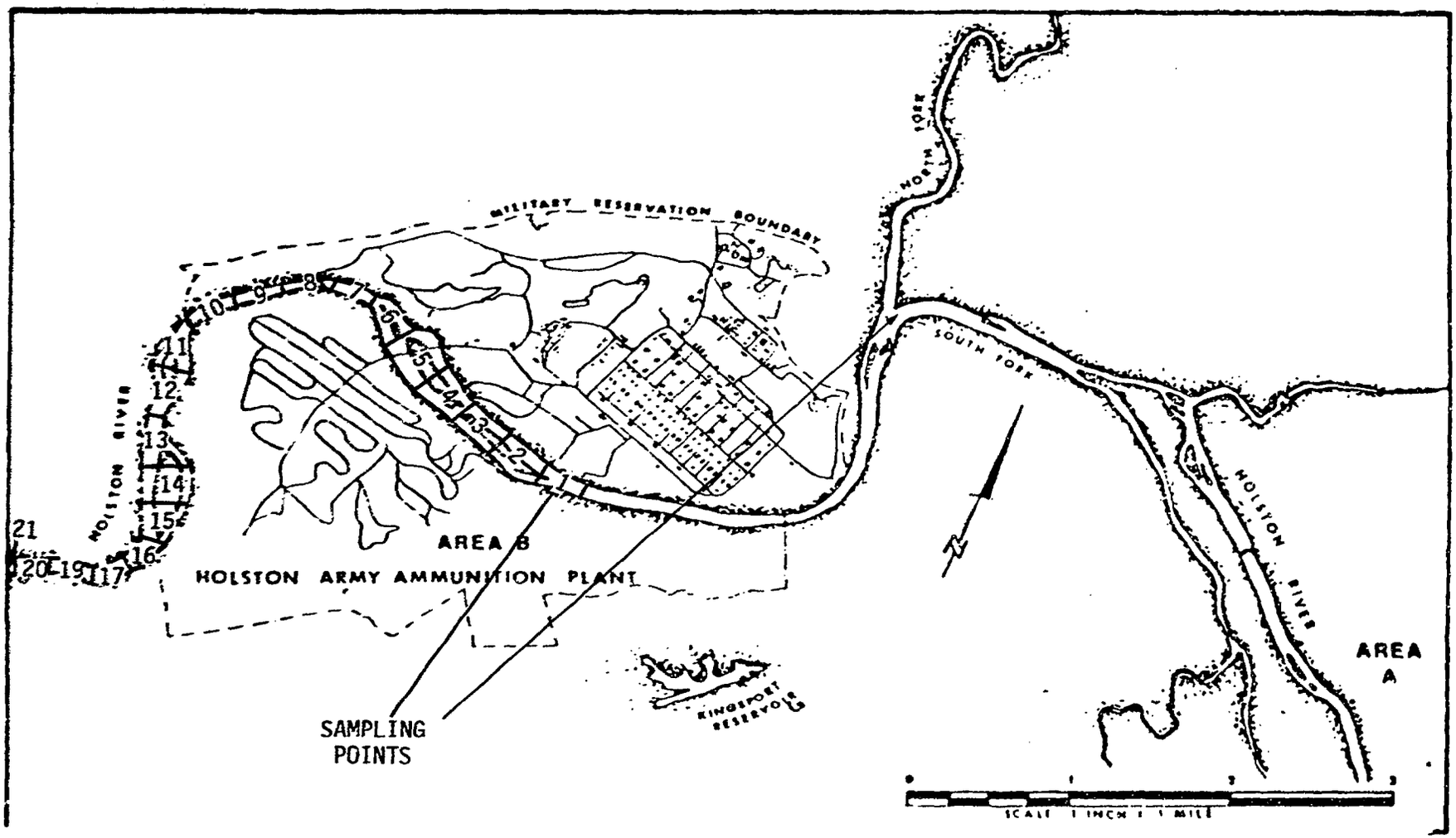


FIGURE A-2 SAMPLING POINTS, HOLSTON AAP

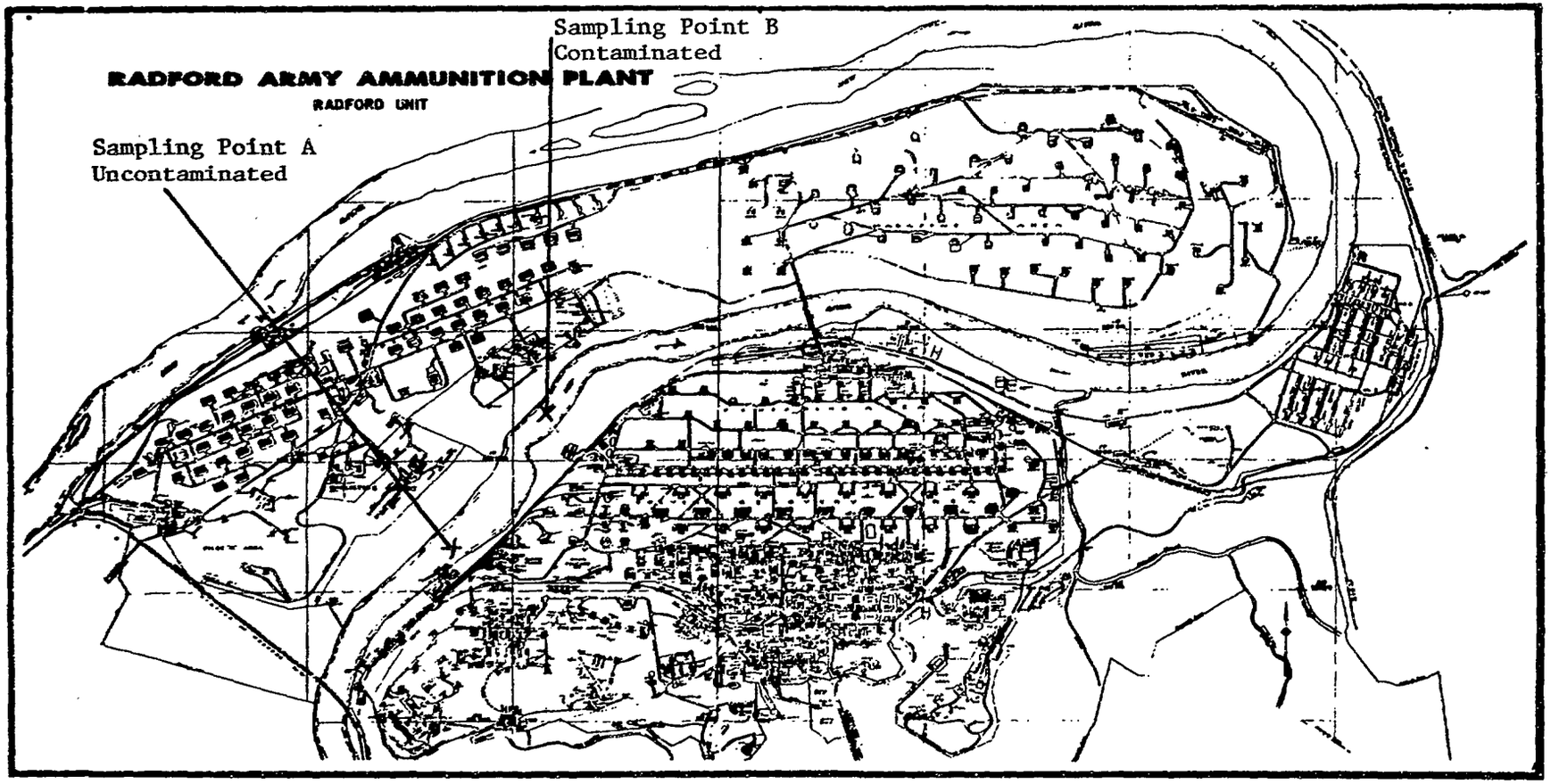


FIGURE A-3 TNC SAMPLING POINTS, RADFORD AAP

at 20 mg ml^{-1}, was added to make a 10 ppm final concentration. Later, DMSO-free sterile basal-salts medium containing 100 µg TNT ml^{-1} was used as the stock solution. TNG was extracted from a 10% TNG on lactose formulation (ICN Chemicals) with acetone, evaporated to an oil, and diluted with water to yield a 700-ppm aqueous solution. Yeast extract was added to the sterile medium from a 25 mg ml^{-1} sterile aqueous solution.

The bottle was fitted for sterile aeration, air exhaust, sampling, and addition of other nutrients. The bottles were aerated gently at 25°C in a constant-temperature room in the dark. For anaerobic (microaerophilic) studies, 2 liters of water sample and 20 ml of buffer were placed in a 2-liter Erlenmeyer flask, flushed with N_2 gas, and closed with a rubber stopper. The bottles were shaken in the beginning of the experiment and before every sampling. A red lamp was used during the withdrawal and handling of munition samples. The samples were withdrawn from the bottles periodically and extracted twice with equal volumes of ethyl acetate or used directly for chemical analysis.

The cultures were transferred to flasks containing test chemical and basal-salts media. The phosphate-buffer basal-salts medium contained, per liter: 1.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of NaCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, and 1 ml of trace elements solution. The trace elements solution contained, per liter: 0.1 g of H_3BO_3 and 0.05 g each of $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6H_2O$.

The flasks were incubated on a rotary shaker incubator at 25°C in the dark.

To preserve TNT-yeast-extract-degrading microorganisms, water from the TNT-screening test bottle was inoculated into basal-salts medium containing 10 ppm TNT and 1000 ppm yeast extract, and the grown cells were centrifuged, resuspended in 5% DMSO solution, and stored in the gas phase of a liquid nitrogen storage tank. The 2,4-DNT and TNG

degrading organisms were grown in basal-salts medium containing 100-120 ppm 2,4-DNT or TNG and preserved in the same manner. Before use as the inoculum for experiments, the frozen cells were grown in TNT-yeast extract medium at least once.

3. Ring-Labeled ^{14}C-TNT, ^{14}C-DNT Transformation

The ultimate biotransformation of TNT and 2,4-DNT was investigated with a sealed flask (Figure A-4), modified from that of Gledhill (1975), with uniformly ring-labeled ^{14}C-TNT (Pathfinder Lab., Inc., St. Louis, MO, 5.81 mCi mmole^{-1}) or ring-labeled ^{14}C-2,4-DNT (New England Nuclear, Boston, MA, 2.52 mCi mmole^{-1}). Radiolabeled TNT medium was prepared by adding 0.5 µCi of ^{14}C-TNT in 8 µl of acetone into 50 ml of basal-salts medium containing 10 ppm TNT and 1000 ppm yeast extract. Ring-labeled 2,4-DNT medium was prepared by adding 25 µl of DMSO containing 5 mg of 2,4-DNT and 0.5 µCi of ^{14}C-DNT into 50 ml of basal-salts medium. The 250-ml sealed flask was equipped with a glass tube inside the flask containing 5 ml of 0.5 N KOH solution to trap evolved CO_2. The KOH solution was replaced daily with fresh solution with the aid of a long needle syringe. The head space of the flask was flushed with a 70% O_2-30% N_2 mixture to provide enough oxygen for the microorganisms at the beginning of the study and at every sampling time. The flask was incubated on a shaker at 25°C in the dark. Radioactivity was counted after adding 0.5 ml of KOH solution to a scintillation vial with 10 ml of Scintisol scintillation liquid (Isolab, Akron, OH). The KOH solution was also mixed with equal volumes of 10% $BaCl_2$ solution, and the supernatant was used as a blank for radioactivity count.

4. Monitoring of TNT, RDX, and 2,4-DNT

HPLC was used to monitor TNT, RDX, and 2,4-DNT in biotransformation studies. The following HPLC conditions were employed:

Instrument: Spectra-Physics Model 3500B
Column: C_{18}-Radial Compression Column (Waters Assoc.)
Solvent: Methanol:water (50:50)
Flow Rate: 2 ml min^{-1}

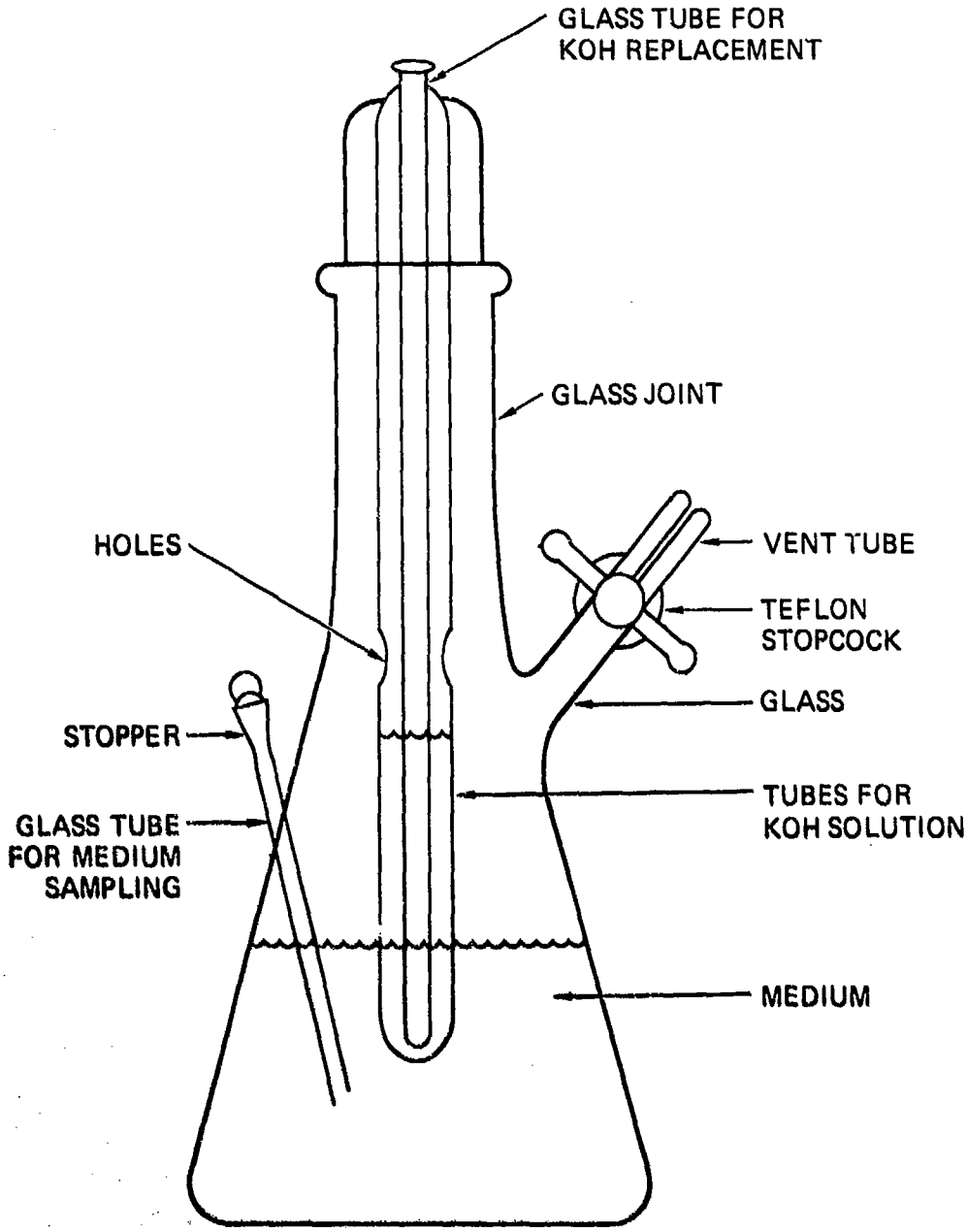


FIGURE A-4 APPARATUS FOR STUDYING BIOTRANSFORMATION

Detector: UV at 254 nm

Retention times: RDX, 200 s
TNT, 350 s
2,4-DNT, 550 s
3,5-DNT, 620 s.

Each component was quantified by using 3,5-dinitrotoluene as the internal standard. Peak areas were integrated with a Spectra-Physics Minigrator digital integrator.

5. TNT and 2,4-DNT Metabolite Studies

Media samples (50-100 ml) were extracted with 2 × 50 ml of ethyl acetate, rotary-evaporated to dryness, and redissolved in methanol. The samples were analyzed by HPLC under the following conditions:

Instrument: Spectra-Physics Model 3500B

Column: C_{18}-Radial Compression Column (Waters Assoc.)

Solvent: Linear gradient program starting from 100% water → 70% methanol in 15 minutes.

Flow rate: 2 ml min^{-1}

Detector: UV at 254 nm.

Eluting components were collected from repetitive injections, concentrated to dryness with a stream of N_2 gas, and analyzed by probe mass spectrometry with an LKB9000 mass spectrometer.

6. TNG Analysis

TNG was analyzed by HPLC using the following conditions:

Instrument: Spectra-Physics Model 3500B

Column: C_{18}-Radial Compression Column (Waters Assoc.)

Solvent: Water:methanol (60:40)

Flow rate: 2 ml min^{-1}

Detector: UV at 215 nm

Retention time: RDX (internal standard), 300 s
TNG, 650 s.

Quantitation was achieved by using RDX as the internal standard. Peak areas were integrated with a Spectra-Physics Minigrator digital integrator.

TNG in natural waters and TNG metabolites were investigated by HPLC using the Thermal Energy Analyzer detector (TEA) according to the method of Spanggord and Keck (1980).

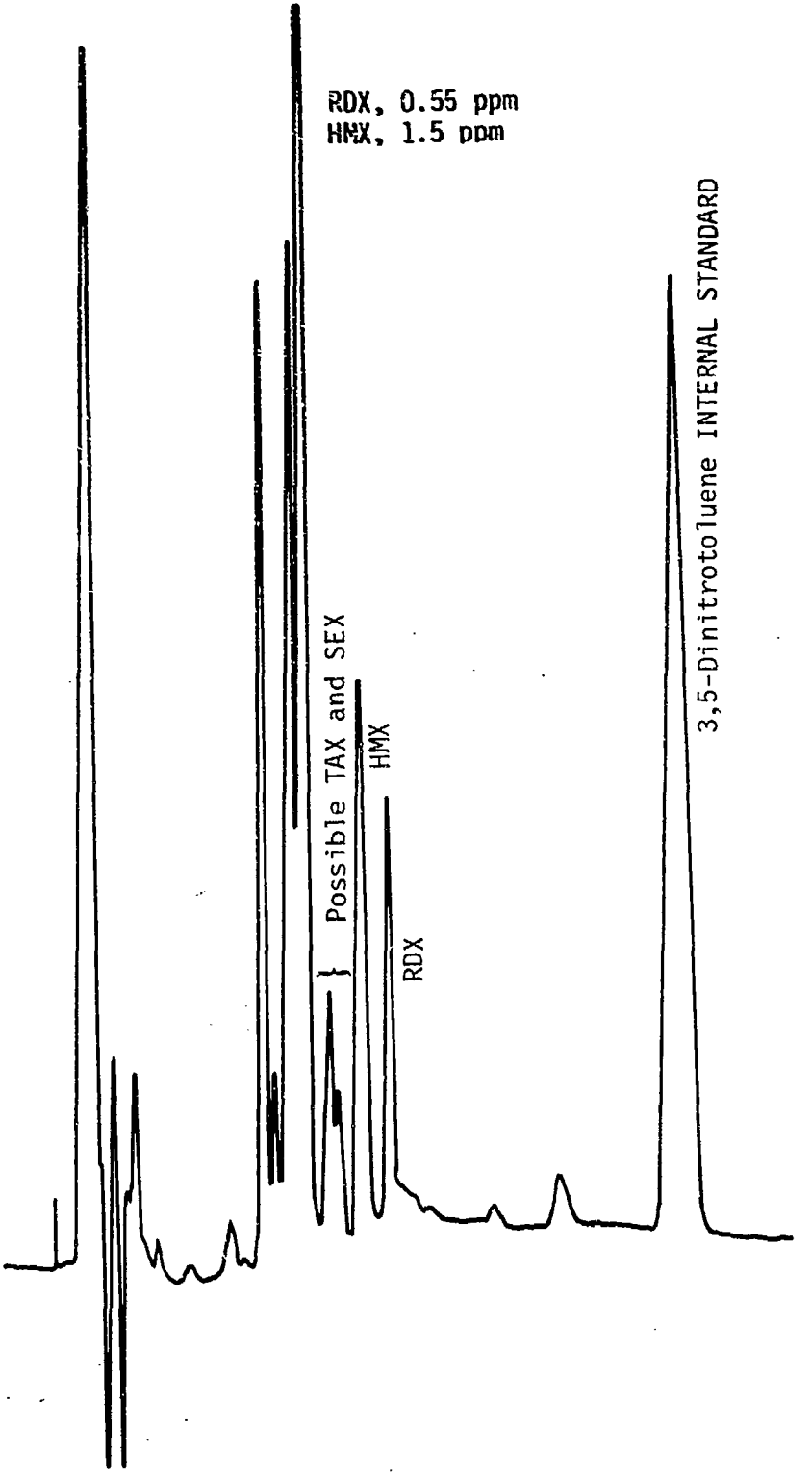
C. Natural Water Analysis

Natural waters were analyzed by HPLC using direct aqueous injection of the filtered water sample under the following conditions:

Instrument: Spectra-Physics Model 3500B
Column: C_{18}-Radial Compression Column (Waters Assoc.)
Solvent: 100% water → 40% methanol:acetonitrile (2:1) in 10 min using a convex gradient
Flow rate: 2 ml min^{-1}
Detector: UV at 254 nm
Retention time: HMX, 714 s
RDX, 774 s.

Profiles of the Holston River samplings appear in Figures A-5 and A-6. The compounds RDX, HMX, SEX, and TAX were identified by comparing the retention times to those of authentic standards. RDX and HMX were quantified by using 3,5-dinitrotoluene as the internal standard. In the July 1979 sampling, 0.55 ppm of RDX and 1.5 ppm of HMX were found, and in the August 1979 sampling, 0.31 ppm of RDX and 0.52 ppm of HMX were found.

The New River and Waconda Bay waters showed no UV-absorbing compounds by direct aqueous injection. TNT and 2,4-DNT concentrations were therefore below 30 ppb in those water bodies.

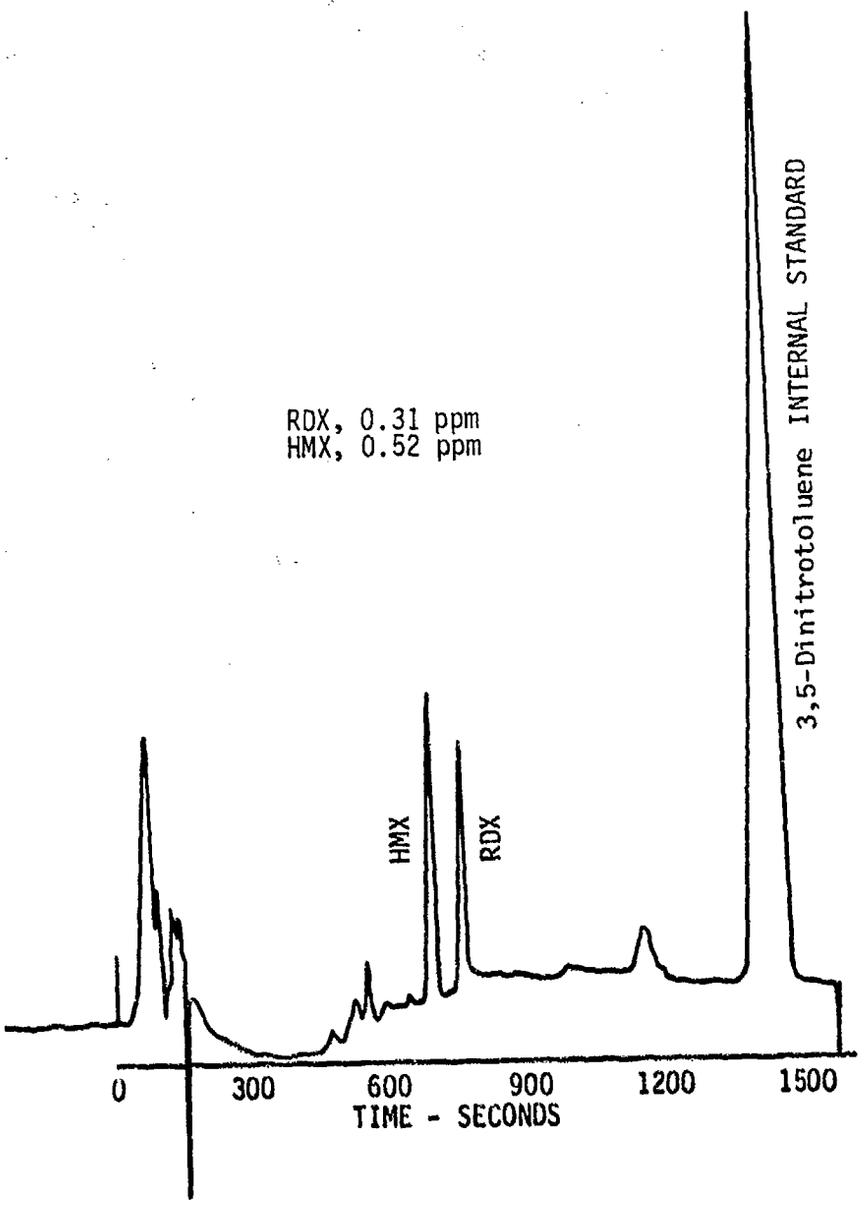


HMX = Octahydro-1,3,5,7-Tetranitro-1,3,5,7--tetrazocine

TAX = Hexahydro-1-Acetyl-3,5-dinitro-1,3,5-triazine

SEX = Octahydro-1-Acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine

FIGURE A-5 HPLC PROFILE OF HOLSTON RIVER WATER COLLECTED ON JULY 18, 1979



HMX = Octahydro-1,3,5,7-Tetranitro-1,3,5,7-tetrazocine

FIGURE A-6 HPLC PROFILE OF HOLSTON RIVER WATER COLLECTED ON AUGUST 7, 1979

For TNG in the New River, 340 ml of river water was extracted 3 times with 50-ml portions of dichloromethane. The extracts were combined, dried, and rotary evaporated to 100 µl and analyzed by the HPLC method of Spanggord and Keck (1980). No TNG or its hydrolysis products were observed.

D. Biosorption Studies

The biosorption study was conducted with mixtures of 4 species of gram-positive and gram-negative aquatic-origin bacteria. The mixture contained equal optical densities of Azotobactor beijerinckii ATCC 19366, Bacillus cereus ATCC 11778, Escherichia coli ATCC 9637, and Serratia marcescens ATCC 13880.

The test organisms were grown in Trypticase Soy broth at 25°C for 16-18 hours, and were in the late logarithmic or early stationary growth phases. Each culture was harvested by centrifuging, washed with 0.5 g liter^{-1} potassium phosphate buffer (pH 7.0), and resuspended with this buffer. No spore formation of Bacillus cereus was observed by microscopic examination.

Appropriate aliquots of suspension of each of the 4 organisms were combined and diluted with buffer to form a mixture containing equal optical densities of each organism. When heat-killed cells were used, the mixture of organisms was heated for 20 minutes in a boiling water bath, centrifuged, and resuspended in phosphate buffer. The mixture was then mixed with a chemical stock solution of 50-100 ppm in buffer to make the desired concentration of chemical and organisms. Triplicate 10-ml samples of each of the cells and chemical mixtures were dispensed in 30-ml Corex centrifuge tubes.

Biosorption studies were conducted by incubating the cell mixtures for 1 hour at 25°C on a reciprocate shaker. The tubes were centrifuged, the supernatants were pipetted, and the pellets were extracted with ethyl acetate. The chemical in the supernatants and pellets was analyzed by HPLC.

Dry weights of the cells used in the sorption studies were determined by weighing the pellet obtained after cells from aliquots of mixed cells were centrifuged and dried at 90°C.

The biosorption partition coefficient (K_p) of the chemical between bacteria and buffer was determined as:

$$K_p = \frac{\mu g \text{ chemical per g dry weight of cells}}{\mu g \text{ chemical per ml in supernatant}}$$

E. Sediment Sorption Studies

1. Sediment Collection, Preparation, and Storage

Sediment was collected from sites on the Holston River and from Waconda Bay in Tennessee near the wastewater discharge of 2 munitions plants in July and August 1979. Sediment was taken both upstream and downstream of the discharge point. The sediment was placed in 1-gallon (3.8-liter) glass containers and shipped to SRI.

The procedures used to prepare and store the natural sediments were designed to preserve the sediments in their natural state as well as possible. Natural sediments were screened through a 2-mm sieve to remove large rocks, twigs, and other debris. Following this screening the sediments were thoroughly mixed, using a Humbolt splitter to ensure uniformity. The sediment was then stored in a refrigerator in one-gallon glass containers until needed. Before use, the sediments were resuspended in water and used without further settling.

After several sorption isotherms were measured for TNT on Holston River (upstream) sediment, it was discovered that biologic organisms in the sediment were biodegrading the TNT. Sediment sterilized in an autoclave was used in subsequent TNT sorption isotherm studies and in all the sorption isotherm studies with 2,4-DNT, RDX, and TNG. After it was autoclaved at 120°C for 1 hour, the sediment was removed and stored at room temperature for 24 hours. The sediment was mixed and then autoclaved again for 1 hour. The sterilized sediment was stored in a refrigerator until needed.

2. Organic Carbon Content Analysis

The organic carbon (OC) content of the sediments was determined by the Walkley and Black procedure, which involves oxidation of the organic material by chromate followed by back-titration with ferrous ammonium sulfate (Hesse, 1971). The results are reported in Table A-1 and expressed as percent carbon by weight. The organic content was measured for the sediments that had been screened, suspended in water, and allowed to settle for 30 seconds to remove particles larger than 100 μm. The OC of the samples of the whole Holston River (upstream) sediment was also measured before it was suspended in water and allowed to settle. Other methods of determining OC values, such as combustion to CO_2, probably would give different OC values. However, the trend in the organic carbon (OC) levels in the sediments should not change.

Table A-1

ORGANIC CARBON CONTENT OF SEDIMENTS

Source	Preparation	OC (%)
Holston River (upstream)	Unsettled fraction	4.7
Holston River (downstream)	Unsettled fraction	2.0
Waconda Bay (upstream)	Unsettled fraction	2.3
Waconda Bay (downstream)	Unsettled fraction	1.1
Holston River (upstream)	Whole sediment	3.3

Solutions of the compounds of interest were prepared in distilled deionized (DDI) water. Concentrations of the stock solutions were approximately half the solubility achievable in water or less to avoid the presence of undissolved compound during the measurement of the sorption isotherm.

Sediment from the Holston River collected at the upstream site was used for all the isotherm measurements in this study. The amount of sediment was measured by evaporating a known volume of sediment suspension and drying it to constant weight at 120°C. Deionized water was used to bring each flask to a constant volume. In some isotherms, $HgCl_2$ was added to retard biodegradation.

In general, the sorption isotherms were measured by preparing flasks containing only the compound in water, only sediment and water, and both the compound and sediment. For screening isotherms, 2 different concentrations of sediment were added to 1 concentration of the compound. The conditions selected for the detailed isotherm measurements were based on the results of the screening isotherm measurement by using the value of K_{oc} measured in the screening isotherm to calculate the amount of compound and sediment needed to achieve about 50% sorption at equilibrium. The sediments used for the desorption isotherms were prepared by resuspending sediment that had been equilibrated for 200-300 hours with the compound in fresh DDI water.

The flasks were wrapped in aluminum foil to exclude light, capped with a glass stopper, and placed on a wrist-action shaker set for gentle shaking. Samples from the flasks were removed just after preparation and again after equilibrium had been achieved between the compound in solution and in the sediment. When the rate of approach to equilibrium was measured, samples were taken at regular intervals until equilibrium was achieved.

3. Sorption Experiments with TNT

Aliquots of the isotherm suspension were centrifuged at 10,000 rpm for 30 minutes (equivalent to approximately 8×10^3 rcf for the centrifuge head and tubes that were used). The supernatant and sediment were separated. The supernatant was filtered before analysis by HPLC through 5 μm Millipore teflon filters. The sediment was extracted 3 times with ethyl acetate. After the extracts were combined and made up to a known volume, the resulting suspension was filtered and analyzed by HPLC.

Two HPLC analytical methods were used in the analysis of TNT isotherms. The first method was used to analyze the screening and first sorption-desorption isotherm. Instrumental conditions were:

Instrument: Model 848 Dupont HPLC
Column: μ Bondapak C_{18} (Waters Assoc.), 20 cm × 4 mm
Solvent: Acetonitrile:water (50:50)
Flow rate: 2 ml min^{-1}
Detector: UV at 254 nm
External standard: TNT.

The second method was used to measure the second sorption-desorption and ^{14}C sorption isotherms. The following instrumentation and conditions were used:

Instrument: Spectra-Physics 3500B HPLC
Column: C_{18} RCM-100 (Waters Assoc.)
Solvent: Methanol:water (50:50)
Flow rate: 2 ml min^{-1}
Detector: UV at 254 nm
Internal standard: 2,4-DNT

The sorption experiments with ^{14}C-TNT were performed in the following manner. A solution of 65 mg l^{-1} TNT and 0.9 mg l^{-1} ring-labeled ^{14}C-TNT (Pathfinder Lab., Inc., St. Louis, Mo., 5.81 mCi $mmole^{-1}$) was prepared for the sorption study. The solution was equilibrated with sediment. The separation of the supernatant and the sediment and the extraction of the sediment were performed in the same manner as for all sorption experiments. Supernatants and sediment extracts were analyzed by liquid scintillation counting using a Tri-Car 6 oxidizer.

4. Sorption Experiments with RDX

Supernatants and sediment extracts were analyzed by HPLC. The specific conditions used for the analysis of RDX are described below.

Instrument: Spectra-Physics 3500B HPLC

Column: C_{18}-RCM-100 (Waters Assoc.)

Solvent: Methanol:water (50:50)

Flow rate: 2 ml min^{-1}

Detector: UV at 215 nm

Internal standard: 3,5-DNT.

5. Sorption Experiments with 2,4-DNT

Supernatants and sediment extracts were analyzed by HPLC. Conditions used for the analysis of 2,4-DNT were as follows:

Instrument: Spectra-Physics 3500B HPLC

Column: C_{18}-RCM-100 (Waters Assoc.)

Mobile phase: Methanol:water (50:50)

Flow Rate: 2 ml min^{-1}

Detector: UV at 254 nm

Internal standard: 3,5-DNT.

6. Sorption Experiments with TNG

Supernatant and sediment extracts were analyzed by HPLC. The specific conditions used for the analysis of TNG in these samples are listed below.

Instrument: Spectra-Physics 3500B HPLC

Column: C_{18}-RCM-100 (Waters Assoc.)

Solvent: Methanol:Water (40:60)

Flow rate: 2 ml min^{-1}

Detector: UV at 215 nm

Internal standard: RDX.

F. 2,4-DNT Volatilization Experiment

A solution of 2,4-DNT in water was placed in a 2-liter crystallizing dish. The stirring rate of the solution and the air velocity above the solution was adjusted to a desired value and kept constant during the

experiment. The loss of 2,4-DNT was measured at intervals for 1 half-life. The loss of water, from which the water-loss rate constant, (k^W) can be calculated, the reaeration rate constant (k_v^O), and the liquid depth (L) and temperature (T) were measured periodically over this time.

The concentration of 2,4-DNT in solution was measured by HPLC with aqueous injection. Dissolved oxygen concentration was measured using dissolved oxygen meter and probe. Water loss was measured by weight, using a triple beam balance.

Conditions used in the HPLC analysis of 2,4-DNT were:

Instrument: Spectra-Physics 3500B HPLC
Column: m Bondapak C_{18} (Waters Assoc.), 300 mm × 4.5 mm
Mobile phase: methanol:water (65:35)
Detector: UV at 254 nm
External standard: 2,4-DNT.

Dissolved oxygen measurements were made on a Bendix Monitor dissolved oxygen-temperature meter and probe.

G. References

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APPENDIX B

SITE-SPECIFIC INPUT PARAMETERS FOR THE SRI MODEL

A. Holston Army Ammunition Plant

Site description. The Holston AAP is located near Kingsport, Tennessee, on the Holston River (Figure B-1). The complex has been divided into two sections: Area A occupies 46 hectares (112 acres) approximately 6.4 km below the Ft. Patrick Henry Dam on the north bank of the river; Area B occupies 2424 hectares (5,913 acres) of land along about 6.4 km of the river. RDX, TNT, and other constituents involved in munitions manufacture are used at the facility in Area B. The wastes from Area B are discharged into the Holston River, as shown in Figure B-2. Munitions wastes from points 2, 3, and 4 are discharged into small creeks and then drain southward to the main body of the Holston River. These creeks are approximately 0.6 to 0.9 m wide and 0.3 m deep.

Other industries are located upstream of the Holston AAP and also contribute industrial discharges into the Holston River. Consequently, water quality near the studied area may influence the rates of munition transformation processes in the Holston River. Distinct segregation is observed at the confluence point during periods of mean flow. In addition, several small islands located in the middle of the river reach keep the river water even more segregated.

Flow characteristics of the Holston River. In-stream flows of the Holston River near Kingsport are regulated by the Ft. Patrick Henry hydroelectric dam, which is upstream from the AAP. Normally the dam operates to generate electricity for 1 hour in every 4-hour period. Therefore, one would expect the river flow rate to increase every 3 hours. During a rainfall period, it is expected that high flows are released from the dam for flood control.

Flow records from sites near the Holston AAP area are presented in Table B-1. In the present study, a mean-flow rate of 10^5 liters s^{-1} and a low-flow rate of 2.0×10^4 liters s^{-1} were assumed. Generally, good mixing and high dilution in the river are observed during a

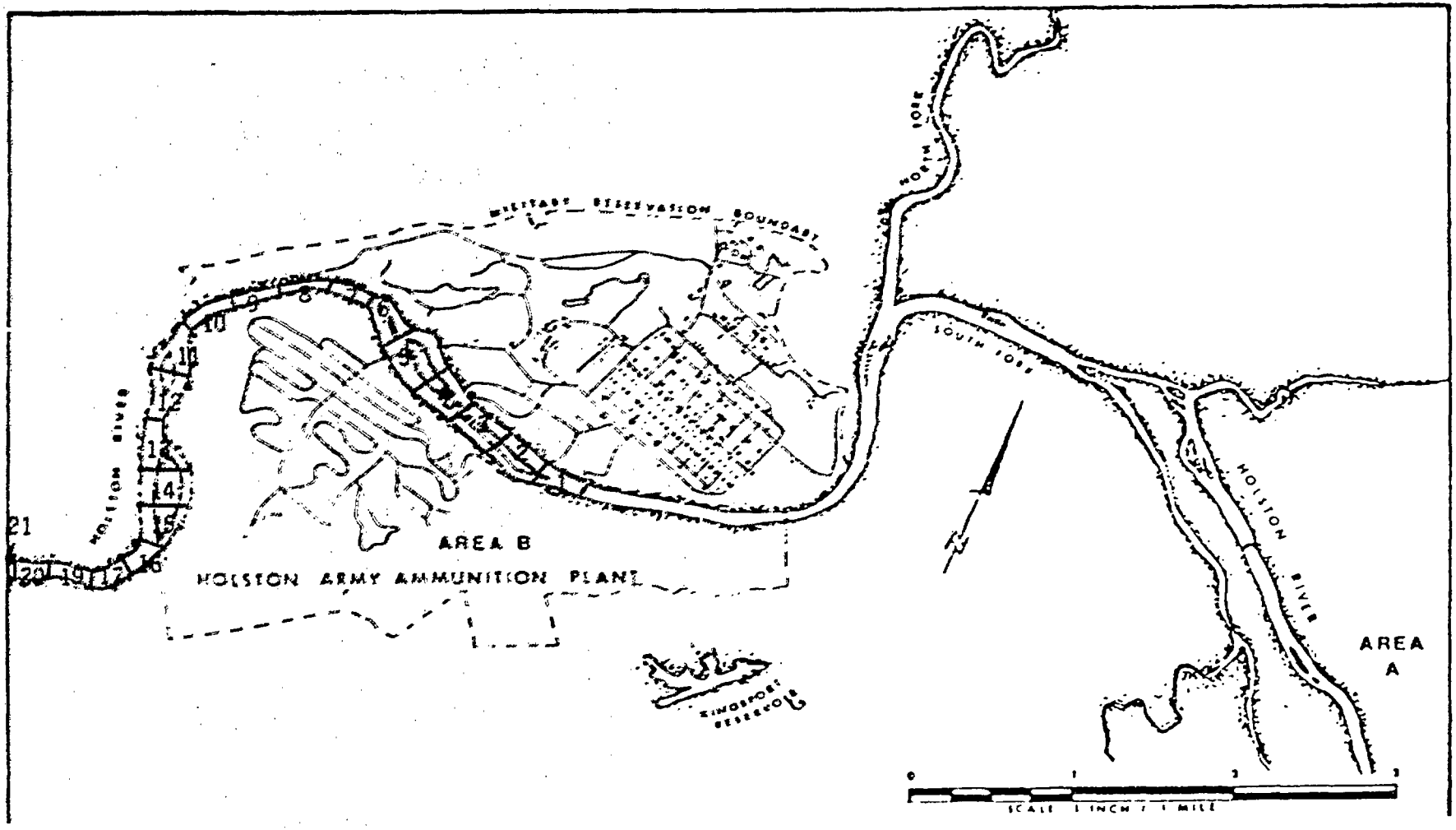


FIGURE B-1 VICINITY OF HOLSTON AAP STUDY AREA AND COMPARTMENTALIZATION OF HOLSTON RIVER

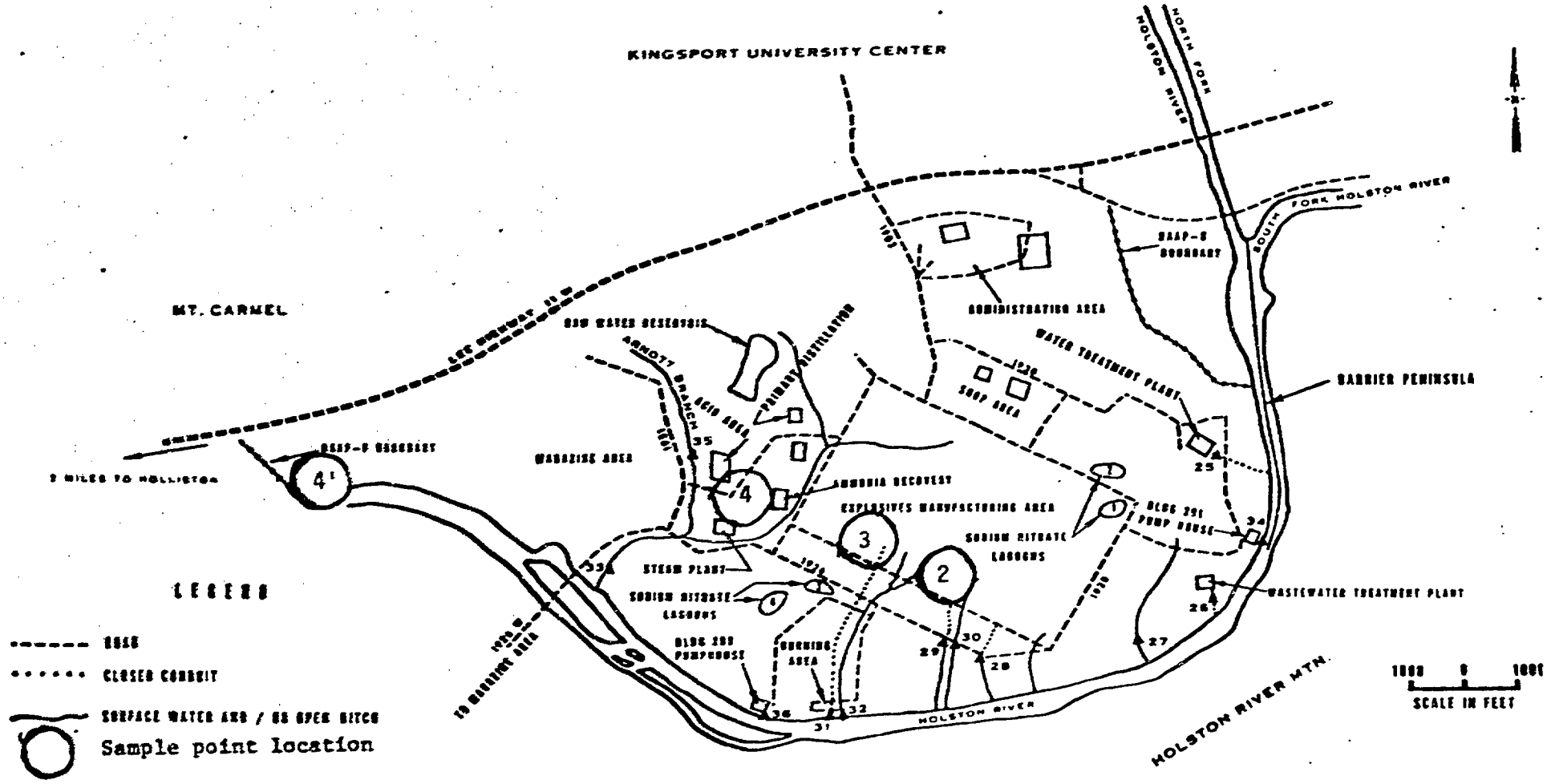


FIGURE B-2 . HOLSTON AAP - GENERAL LOCATION OF WASTE STREAMS

Table B-1

FLOWS OF STREAMS IN THE HOLSTON AAP AREA*

Location: Kingsport, TN

River: South Fork, Holston River

Distance from Holston AAP Area B: 8 km upstream

Year: 1970

High flow:	2.4×10^5 liters s^{-1} (5,5570 MGD)
Low flow:	2.0×10^4 liters s^{-1} (470 MGD)
Mean flow:	5.7×10^4 liters s^{-1} (1,290 MGD)

Historical Data

Mean flow, 45 years of record:	7.1×10^4 liters s^{-1} (1,630 MGD)
High flow, 1963:	6.6×10^5 liters s^{-1} (15,000 MGD)
Low flow, 1954:	8.5×10^3 liters s^{-1} (195 MGD)

*Source: Rosenblatt et al., 1973.

high-flow period. Any environmental problems caused by munition chemicals that have short half-lives will be minimal during high-flow periods.

A dispersion study by Water and Air Research (WAR), Inc. (Sullivan, 1977b) indicated that the discharged trace chemicals in the Holston River do not disperse well and stay on one side of the river bank for several kilometers downstream from the discharge point before being dispersed throughout the transect of the river at a flow rate slightly below the mean flow rate. Therefore, poor dispersion in the Holston River could become the key factor in the assessment of the effects of the discharged munition chemicals.

Water quality. Several water quality monitoring programs have been conducted (Sullivan, 1977b; Rosenblatt, 1973). These programs have shown that the upstream industrial discharges play an important part in water quality in the Holston River near the Holston AAP. Table B-2 shows temperature and pH of the Holston river near the Holston AAP. Figure B-3 shows the dissolved oxygen (DO) profile in the same general area. Suspended solids measured in June and August of 1975 indicated that the concentrations were in the range of 20 to 40 mg $liter^{-1}$. High biological oxygen demand (BOD) loading from the upstream industries, plus no serious DO deficit in that particular river reach, indicate that microbial activity is probably high. Although no detailed data on microbial population are available, it is reasonable to assume that the microbial population is 10^6 cells ml^{-1} near the studied river reach.

Compartments selected for the simulation model. The Holston River near the munition plant was divided into 21 compartments, each 0.4 km long. The simulated river reach represents the river starting from the discharge point of production lines 1 to 5 at Area B of the Holston AAP to about 8.4 km downstream from the discharge point (Figure B-1). The estimated geographic information of each compartment is presented in Tables B-3 and B-4, for the mean flow of 10^9 liters s^{-1} and the low flow of 2×10^6 liters s^{-1}, respectively. In model simulation, the volume of each compartment was assumed to remain constant.

Table B-2

TEMPERATURE AND pH OF THE HOLSTON RIVER NEAR THE HOLSTON AAP[a]

Station	Area B Inlet	Church Hill Bridge	Rogersville, TN
Location	Area B, Holston AAP	16 km downstream	56 km downstream
Time	Jan-Oct 1972	Jan-Oct 1972	Jan-Dec 1970
Temperature Range (°C)	6-22	5-22	0.5-29.0
pH Range (units)	6.4-7.4	6.9-7.0	--

[a]Source: Rosenblatt et al., 1973.

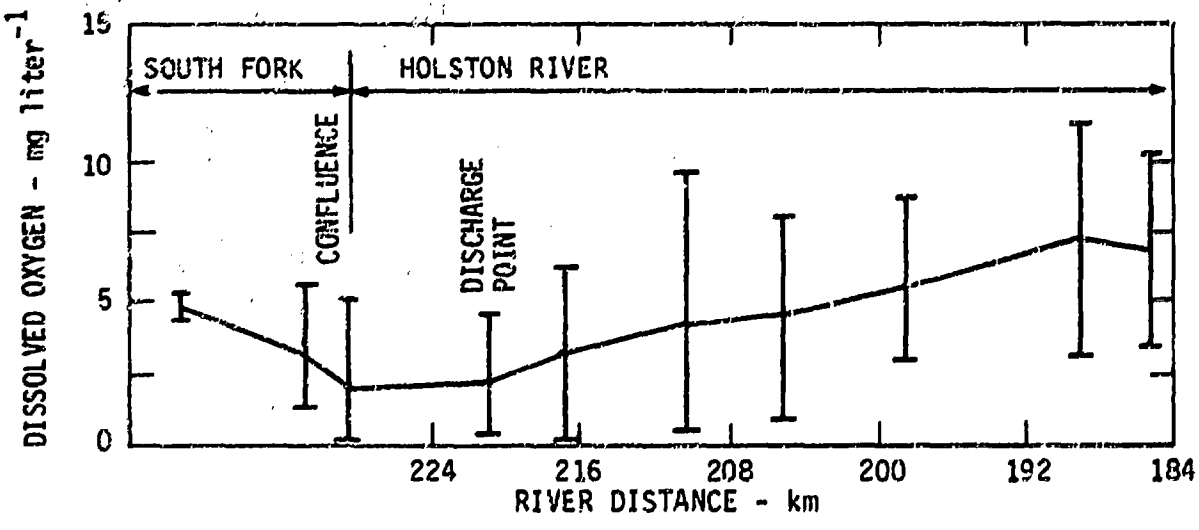


FIGURE B-3 DISSOLVED OXYGEN PROFILE IN THE HOLSTON RIVER

Table B-3

HYDROLOGIC PROPERTIES OF THE HOLSTON RIVER NEAR THE HOLSTON AAP AREA, MEAN FLOW OF 10^5 LITERS s^{-1}

Compartment Number	Cross-Section Area (m^2)	Length (m)	Volume (liters)
1	165.3	400	6.6×10^7
2	206.6	400	8.3×10^7
3	206.6	400	8.3×10^7
4	179.1	400	7.2×10^7
5	206.6	400	8.2×10^7
6	206.6	400	8.3×10^7
7	165.3	400	6.6×10^7
8	128.6	400	5.1×10^7
9	128.6	400	5.1×10^7
10	128.6	400	5.1×10^7
11	128.6	400	5.1×10^7
12	146.9	400	5.8×10^7
13	206.6	400	8.3×10^7
14	206.6	400	8.2×10^7
15	179.1	400	7.2×10^7
16	165.3	400	6.6×10^7
17	165.3	400	6.6×10^7
18	165.3	400	6.6×10^7
19	169.9	400	6.8×10^7
20	169.9	400	6.8×10^7
21	169.9	400	6.8×10^7

Table B-4

HYDROLOGIC PROPERTIES OF THE HOLSTON RIVER NEAR THE HOLSTON AAP AREA, LOW FLOW OF 2.8×10^4 liters s^{-1}

Compartment Number	Cross-Section Area (m^2)	Length (m)	Volume (liters)
1	68.9	400	2.7×10^6
2	86.3	400	3.4×10^6
3	86.3	400	3.4×10^6
4	74.4	400	2.9×10^6
5	86.3	400	3.4×10^6
6	86.3	400	3.4×10^6
7	68.9	400	2.7×10^6
8	45.9	400	1.8×10^6
9	45.9	400	1.8×10^6
10	45.9	400	1.8×10^6
11	45.9	400	1.8×10^6
12	50.5	400	2.2×10^6
13	86.3	400	3.4×10^6
14	86.3	400	3.4×10^6
15	74.4	400	2.9×10^6
16	55.1	400	2.2×10^6
17	55.1	400	2.2×10^6
18	55.1	400	2.2×10^6
19	59.7	400	2.3×10^6
20	59.7	400	2.3×10^6
21	59.7	400	2.3×10^6

Chemical loading at the Holston AAP and the Holston River. Over 34 million kg of TNT were used in munition manufacturing at the Holston Plant in 1970 (Rosenblatt, 1973). An estimate of 73 kg per day of TNT discharged into the Holston River by the Holston AAP was reported by USAEHA survey and Holston Defense Corporation (Stidham, 1979). This was estimated from a discharge of approximately 1,100 liters s^{-1} (25 MGD) from two outfalls [five outfalls fro Area B with a total discharge of 3,564 liters per second (81 MGD)]. The TNT concentration was estimated to be 0.76 mg $liter^{-1}$ in the discharge lines at the plant. The discharge waste water traveling from the plant site to the discharge point at the Holston River takes approximately one hour. Therefore, the TNT concentration at the discharge point was estimated to be 0.2 mg $liter^{-1}$. When compared to the TNT concentrations measured at sample point 3 (Figure B-2), (from 0.2 to 2.01 mg $liter^{-1}$) the TNT concentration estimated at the discharge point is at the low end of this range. For the model study, the TNT loading at the discharge point was estimated to be 0.63×10^3 mg s^{-1}.

Estimate of RDX loading from the Holston Plant. The Holston AAP is the only plant among the 3 studied sites at which RDX is manufactured. Monitoring flows and RDX concentrations in the wastewater streams and the Holston River are shown in Table B-5. RDX discharged from sampling point 4 (Figure B-2) was shown to be relatively low in comparison to the other 2 sampling points. If RDX discharged from points 2 and 3 were the 2 plant effluents out of the 5 plant effluents containing RDX, the total RDX discharged from the plant would be 69.5 kg day^{-1} (0.80×10^3 mg s^{-1}). Because photolysis and biodegradation of RDX were determined to be slow, 69.5 kg day^{-1} of RDX was assumed to discharge directly into compartment 1.

Monitoring data in Holston River. RDX monitoring data at various sampling points reported by Holston Defense Corporation (Stidham, 1979) are presented in Table B-6. In the WAR, Inc. survey (Sullivan, 1976), RDX was found not only in the stream effluent but also in the Holston

Table B-5

RDX FLOWS AND RDX CONCENTRATIONS IN WASTESTREAMS AND IN THE HOLSTON RIVER

Date (1979)	Holston River (4') (liters s^{-1}1	Point 2	Point 3	Point 4
22 and 23 May	1.32×10^5	43.5	117.3	50.5
29 and 30 May	1.62×10^5	43.5	86.6	49.2
5 and 6 Jun	1.56×10^5	40.4	60.1	58.4
12 and 13 Jun	1.52×10^5	40.4	47.5	42.2
Average	1.50×10^5	42.0	77.8	50.1
RDX (X_4) (mg $liter^{-1}$)	0.079	2.55	8.97	0.385
RDX (kg day^{-1})		9.24	60.2	1.66

Source: Stidham, 1979.

Table B-6

CONCENTRATIONS OF RDX AT VARIOUS SAMPLING POINTS IN THE HOLSTON RIVER (Units in ppm)[a]

Date (1979)	Sample Point 2[b]	Sample Point 3	Sample Point 4	Sample Point 4'
10 and 11 May	0.83	16.02	0.11	0.039
16 and 17 May	2.50	9.74	0.340	0.053
22 and 23 May	2.19	5.45	0.609	0.020
29 and 30 May	0.63	15.47[c]	0.240	0.215
5 and 6 Jun	4.41	8.53	0.360	0.058
12 and 13 Jun	2.97	6.43	0.330	0.022

[a]Source: Stidham, 1979.

[b]See Figure B-2 for sample location points.

[c]High values attributed to spillage of RDX and wax caused by malfunction of the deluge system at Building I-1 on these dates.

River. Figures B-4 and B-5 show the distribution of RDX in the Holston River. Consistently higher concentrations of RDX were found by the north bank, indicating that the effluents do not disperse rapidly in the river. More RDX was generally in the river during August. Concentrations of RDX by the north bank averaged 50 ppb; concentrations by the south bank were less than 5 ppb.

B. Radford Army Ammunition Plant

Site description. The Radford AAP is located approximately 11 km north of Radford, Virginia and 13 km southwest of Blacksburg, Virginia. TNT and TNG are produced and used here. In addition, 2,4-DNT is used for munitions manufacture.

The major watercourse at the Radford AAP (shown in Figure B-6) is the New River, which borders the main plant area on the west and north, and separates the nitroglycerin plant from the main plant area. The New River is the primary receiving water for most of the discharges from the Radford AAP, including those discharges emanating from the NG #2 area. TNG and 2,4-DNT are discharged into the New River at discharge point B (Figure B-6). TNT is discharged into Stroubles Creek; Stroubles Creek joins the New River at the east boundary of the plant.

Flow characteristics of the New River. Flow records of the New River at 2 gaging stations near the Radford plant are presented in Table B-7. The New River is regulated for power generation by the Clayton Dam, located several kilometers upstream of the Radford AAP. The State of Virginia has no requirements for minimum New River flow, but flow must be sufficient for demands from the Radford AAP and the Blacksburg-Christianburg-VPI Water Authority, both of which draw water a few kilometers downstream of the dam. In this study, the mean flow of 2.0×10^5 liters s^{-1} and the low flow of 10^4 liters s^{-1} were assumed.

Water quality. Water quality data for the New River in the vicinity of the Radford AAP are presented in Table B-8. In view of this water quality, one may conclude that the New River is in fairly

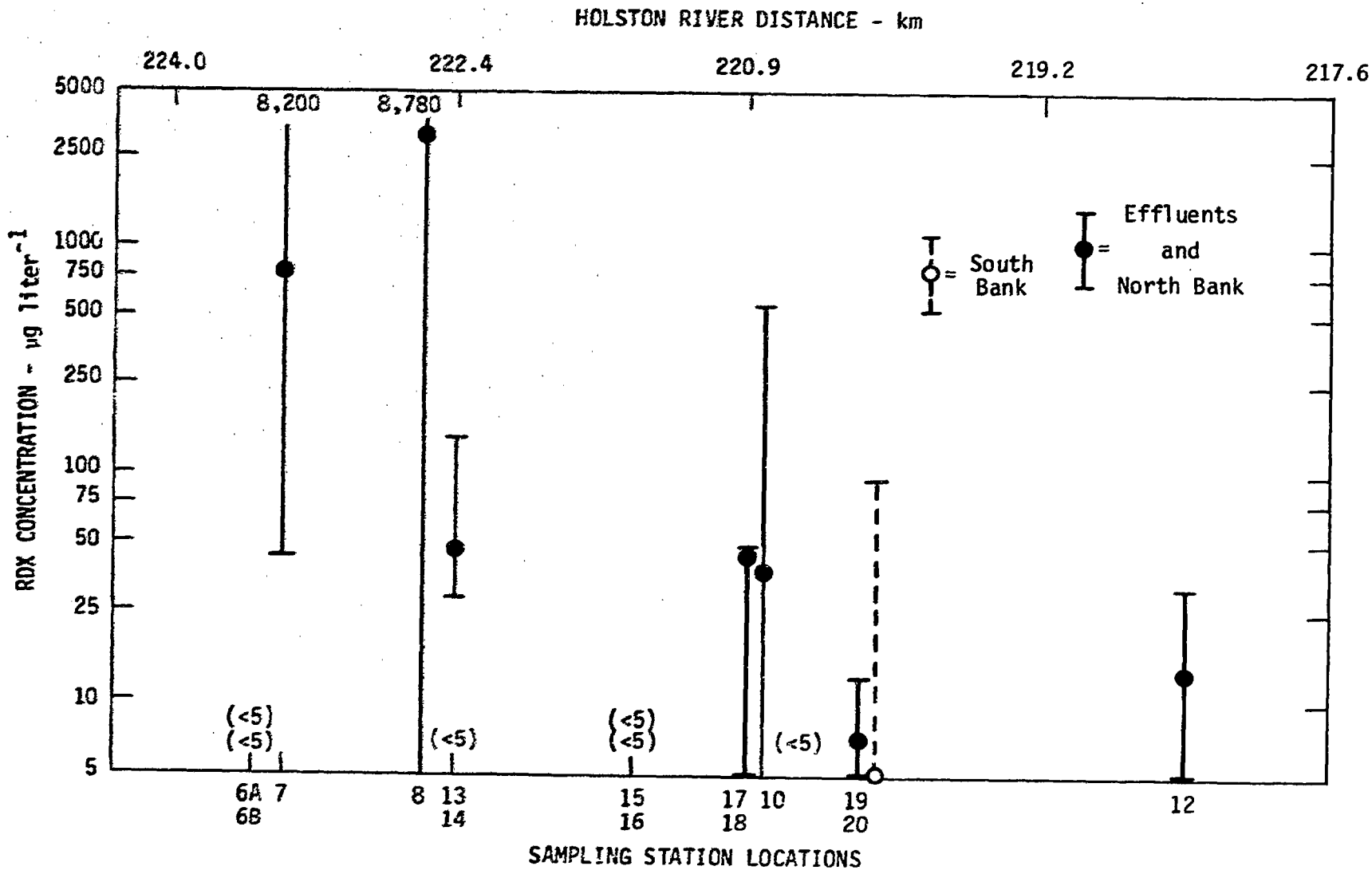


FIGURE B-4 MEDIAN VALUES AND CONCENTRATION RANGE FOR RDX RESIDUES IN THE HOLSTON RIVER, ARNOTT RIVER, ARNOTT BRANCH, AND MUNITIONS EFFLUENTS, JUNE 1975

Source: Sullivan et al., 1977

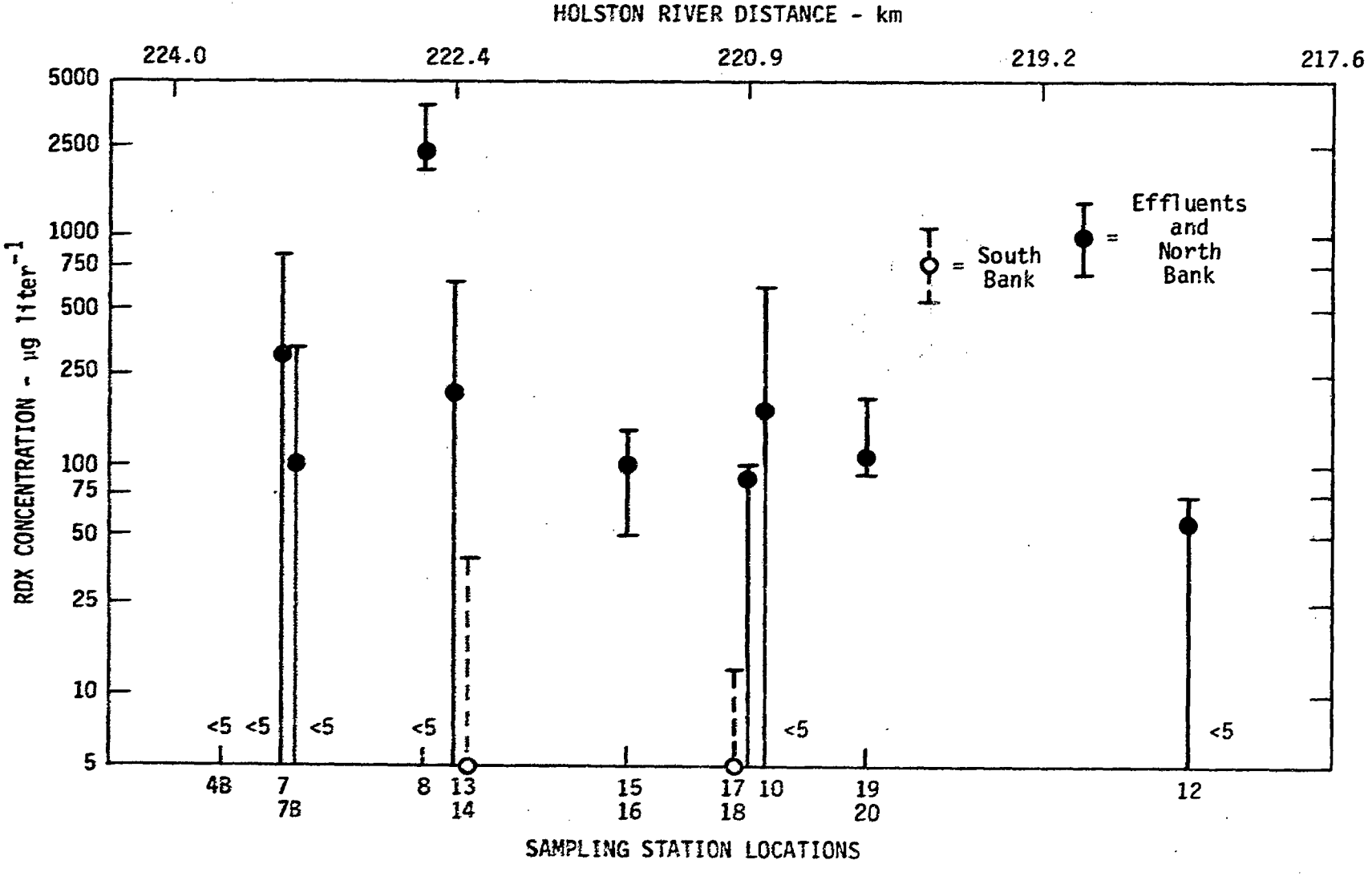


FIGURE B-5 MEDIAN VALUES AND CONCENTRATION RANGE FOR RDX RESIDUES IN THE HOLSTON RIVER, ARNOTT BRANCH, AND MUNITIONS EFFLUENTS, AUGUST 1975

Source: Sullivan et al., 1977

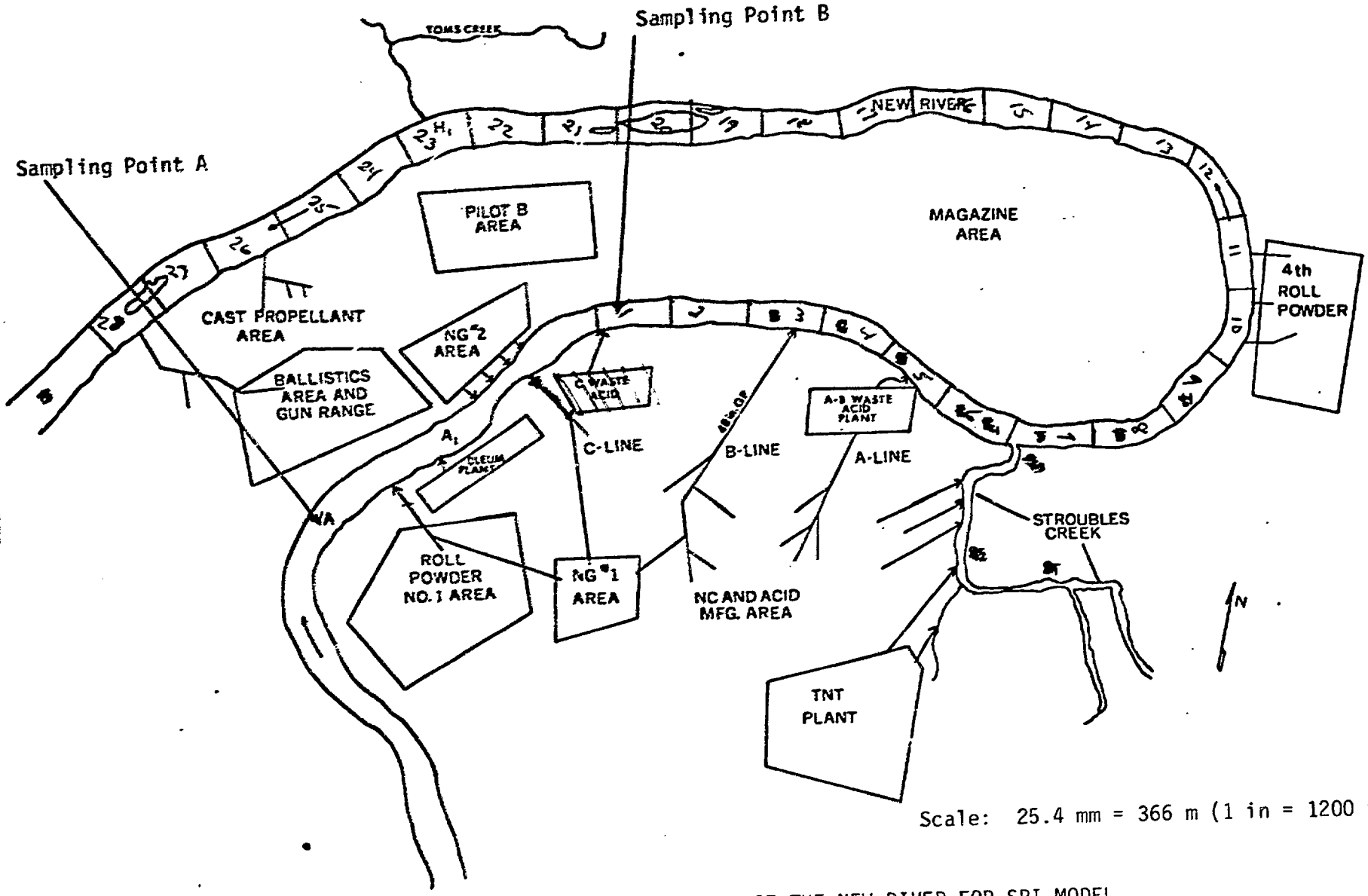


FIGURE B-6 COMPARTMENTALIZATION OF THE NEW RIVER FOR SRI MODEL

Table B-7

FLOWS OF STREAMS IN THE RADFORD AAP AREA*

Location	Radford, VA	Glen Lyn, VA
River	New River	New River
Distance from RAAP	12.8 km upstream	64 km downstream
Water Year	1971	1971
High flow	3.5×10^5 liters s^{-1} (8,140 MGD)	6.5×10^6 liters s^{-1} (14,800 MGD)
Low flow	2.2×10^4 liters s^{-1} (505 MGD)	3.6×10^4 liters s^{-1} (825 MGD)
Mean flow	1.0×10^5 liters s^{-1} (2,380 MGD)	1.4×10^5 liters s^{-1} (3,110 MGD)
Historical Data		
Mean flow	1.0×10^5 liters s^{-1} (2,370 MGD)	1.4×10^5 liters s^{-1} (3,110 MGD)
Years of record	40	44
High flow	6.2×10^6 liters s^{-1} (141,000 MGD)	6.4×10^6 liters s^{-1} (146,000 MGD)
Year	1940	1940
Low flow	4.7×10^3 liters s^{-1} (107 MGD)	2.3×10^4 liters s^{-1} (529 MGD)
Year	1944	1930

*Source: Rosenblatt et al., 1973.

Table B-8

NEW RIVER WATER QUALITY DATA, 16 MAY 1975

Parameter	Units	Stations (See Figure B-7)				
		R1	R3	R4	R5	R6
Specific conductance	μmhos cm^{-1}	60	78	65	82	56
Total solids	mg $liter^{-1}$	94	70	67	83	81
Total suspended solids	mg $liter^{-1}$	4	5	4	4	5
pH	SU	7.05	7.40	7.60	7.55	7.55
Total alkalinity	mg $liter^{-1}$ as $CaCO_3$	37	36	38	38	37
Chloride	mg $liter^{-1}$	4.6	4.8	5.3	5.2	4.7
Sulfate	mg $liter^{-1}$	10	7	7	24	7
Total hardness	mg $liter^{-1}$ as $CaCO_3$	44	41	40	39	39
Calcium	mg $liter^{-1}$	11.3	10.2	9.7	9.4	9.3
Magnesium	mg $liter^{-1}$	3.8	3.8	3.8	3.8	3.8
Sodium	mg $liter^{-1}$	2.4	2.6	2.6	2.5	2.5
Potassium	mg $liter^{-1}$	1.6	1.6	1.6	1.6	1.6
Dissolved oxygen	mg $liter^{-1}$	9.5	9.5	9.4	9.5	9.2
BOD	mg $liter^{-1}$	2	1	1	2	2
Chemical oxygen demand (COC)	mg $liter^{-1}$	7	10	< 5	8	42
Total organic carbon (TOC)	mg $liter^{-1}$	2	4	3	4	40
Kjeldahl-N	mg $liter^{-1}$	0.1	0.2	0.3	0.3	0.3
Ammonia-N	mg $liter^{-1}$	0.070	0.069	0.094	0.073	0.098

Source: Weitzel et al., 1976.

ecologic condition. Both pH and DO are within normal limits. Suspended solids in the studied river reach are less than 10 ppm. Although no microbial population data are available, 10^6 cells ml^{-1} are expected in the river.

Compartments selected for the simulation model. In the vicinity of the Radford AAP, the New River is about 120 to 150 m wide. The main channel is approximately 40 m wide and 1 m to more than 3 m deep over the stretch of river studied. Fourteen km of the river reach, starting from discharge point B (as shown in Figure B-6), were investigated for the model simulation. The studied river reach was divided into 28 equal-sized compartments, with each compartment being approximately 0.5 km long. Because no detailed geographic and hydrologic data for the area near the Radford AAP were available, the average cross-section area of 35 m^2 at the low-flow period and 210 m^2 at the high-flow period were assumed for each compartment. The hydrologic data of the high- and low-flow periods selected for the model simulations are presented in Table B-9

Table B-9

HYDROLOGIC DATA OF THE HIGH AND LOW FLOW PERIODS

Flow	Cross-section Area (m^2)	Compartment Dimensions	
		Length (m)	Volume (m^3)
High (2.0×10^3 liters s^{-1})	210	500	105,000
Low (10^3 liters s^{-1})	35	500	16,500

Chemical discharges at the Radford AAP and the New River. The Radford AAP "pink water" discharges were surveyed previously (Rosenblatt, 1973). The mix stream has an approximate flow rate of 7.14 liters s^{-1} (163,000 gallons per day) with TNT concentrations of about 75 mg $liter^{-1}$. This stream was diluted with about 2.0×10^3 liters s^{-1} (4.5 MGD) cooling water before discharge to Stroubles Creek, which then

was discharged to the New River. About 47.7 kg day^{-1} of TNT was reported to be discharged from the Radford plant. The TNT concentration in Stroubles Creek, after dilution by the cooling water, was estimated to be approximately 2.75 mg $liter^{-1}$. If a traveling time of 1 hour for waste waters flowing from the plant to the confluence point of the New River and Stroubles Creek is assumed, the TNT concentration at the confluence point on a clear day should be 0.64 mg $liter^{-1}$. Therefore, the TNT loading, for model simulations, at the confluence point was calculated to be approximately 0.128×10^3 mg s^{-1} (24 pounds day^{-1}).

Estimate of 2,4-DNT loading from the Radford AAP. In the past, the TNT plant discharged approximately 14.1 liters s^{-1} of wastewater containing 90 to 175 ppm nitrobodies. This is equivalent to 2.4×10^3 mg of nitrobodies being discharged from the plant per second. If 50% of the nitrobodies are 2,4-DNT, the estimated 2,4-DNT loading from the plant is about 1.2×10^3 mg s^{-1}. The 2,4-DNT concentration in the New River was determined by Huff et al. (1975) to be 0.5 mg $liter^{-1}$ at the confluence point of the wastestream and the New River. Therefore, the 2,4-DNT loading at the confluence point can be calculated to be 5.0×10^4 mg s^{-1} during the mean-flow period. This estimate appears to be higher than that based on the figure estimated from nitrobody loading by a factor of 50. One possible explanation is that mixing in the New River may not be as good as expected. Therefore, local high concentrations of 2,4-DNT may be detected in the New River. In this study, the 2,4-DNT loading of 1.2×10^3 mg s^{-1} was assumed to be discharged from the plant.

Estimate of TNG from the Radford AAP. At the Radford AAP, 2 nitroglycerin production facilities are available. They are located at Nitroglycerin Areas #1 and #2 (Figure B-6). Nitroglycerin Area #1 is inactive, but nitroglycerin is manufactured in Nitroglycerin Area #2, which employs the Biazzi process. The current production level is approximately 7,727 kg day^{-1}. The process operates with 1 shift each day. Wastewaters come from mixing tank washouts and floor washdowns. All wastewaters are discharged to the New River without further treatment.

Approximately 1.76 liters s^{-1} of water are used in the process, of which 0.22 liters s^{-1} are contaminated with nitroglycerin. Table B-10 shows the sources and amounts of nitroglycerin discharged from the process. A higher nitroglycerin concentration was observed in the tank

Table B-10

SOURCES AND QUANTITIES OF NITROGLYCERIN DISCHARGED FROM NITROGLYCERIN AREA #2

Source	Flow (liters s^{-1})	Nitroglycerin	
		mg $liter^{-1}$	kg s^{-1}
Nitrator	0.11 (2,500 GPD)	1,300	1.4×10^{-4} (27 lbs day^{-1})
Store house	0.11 (2,500 GPD)	266	0.3×10^{-4} (6 lbs day^{-1})

washout from the nitrator than in the floor washdowns from the storehouse. The total nitroglycerin in the wastewaters from the Radford AAP was estimated to be 1.7×10^{-4} kg s^{-1} day^{-1} (33 pounds). which is discharged to compartment 1 of the studied reach in the New River.

Monitoring data in the New River. Monitoring stations in the New River during the survey by Environmental Control Technology Corporation (Weitzel et al., 1976) are shown in Figure B-7. TNG monitoring data from the survey presented in Table B-11indicate that the TNG level normally ranged between 10 and 1 ppb, with the exception of 290 ppb observed at Station R6 on 15 May 1975.

C. Volunteer Army Ammunition Plant

Site description. Volunteer AAP is a TNT-manufacturing facility northwest of Chattanooga, Tennessee. This facility is a 2,920-hectare contractor-operated government munitions manufacturing plant. Wastewater from the plant drains northward into a series of treatment lagoons and is discharged into the head of Waconda Bay. The size of

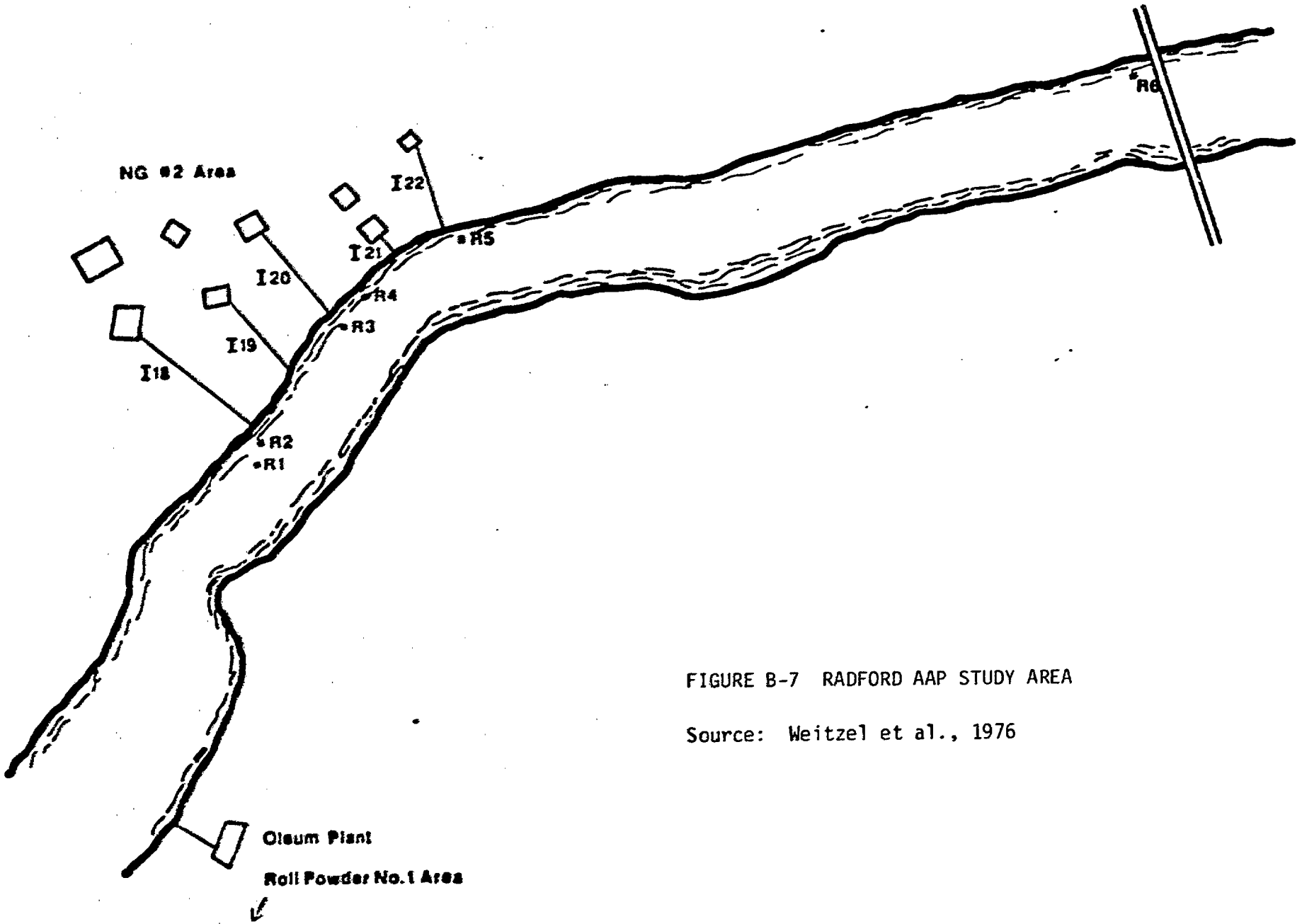


FIGURE B-7 RADFORD AAP STUDY AREA

Source: Weitzel et al., 1976

Table B-11

CONCENTRATIONS (ppm) OF TNG IN THE NEW RIVER

Date, 1975	New River Station (See Figure B-7)					
	R_1	R_2	R_3	R_4	R_5	R_6
15 May	< 0.01	--	--	< 0.01	--	0.29
16 May	0.05	--	< 0.01	0.03	< 0.01	< 0.01
21 May	0.03	--	0.01	< 0.01	< 0.01	< 0.01
30 Oct	0.002	--	--	--	< 0.002	0.003
1 Nov	< 0.002	--	--	--	0.007	--
17 May	--	--	< 0.01	--	< 0.01	--
20 May	< 0.01	--	< 0.01	< 0.01	< 0.01	0.03
22 May	< 0.01	--	< 0.01	< 0.01	< 0.01	< 0.01
31 Oct	0.01	--	--	--	< 0.002	< 0.002

Source: Weitzel et al., 1976.

Waconda Bay is about 94 hectares and the mean depth is about 3 meters. The head of the bay is much shallower than the rest of the bay.

Flushing of Waconda Bay is caused by three factors: (1) runoff, (2) stage fluctuation, and (3) effluent from the plant. The drainage area for Waconda Bay is approximately 9 times the area of the bay. During rainfall periods, surface runoff may contribute significant inflows to the bay. At an effluent flow of 220 liters s^{-1} (5 MGD), Waconda Bay will be about 20 percent flushed in 1 month. This means that the residence time of the plant effluent in Waconda Bay is about 5 months if there are no other inflows. The effectiveness of flushing by stage variation (or intruding water from the Tennessee River) was not investigated, but it is believed to be an important hydrologic process in Waconda Bay. Although no detailed data on the circulation pattern of water in Waconda Bay are available, monitoring data collected in 1975 indicate that the plant effluent tends to travel along the north side of the bay. Furthermore, the water of the Tennessee River coming to Harrison Bay flows into Waconda Bay (Figure B-8). The intruding water apparently moves along the south side of the bay. A clockwise circulation pattern is expected inside of the bay. The circulation pattern also depends on wind direction. Although no field measurements are available, some intrusion of water from the Tennessee River was assumed for model simulation.

Flow characteristics. The Tennessee River is dammed above Chattanooga by the Chickamagua Dam. Flow records taken at a gage station about 4.8 km below the dam showed a mean flow of 1×10^6 liter s^{-1} (23,600 MGD) over a 96-year period with a record low flow noted in 1953 of 3.4×10^4 liter s^{-1} (780 MGD). Approximately 50% of the river water was assumed to flow along the south shore of Harrison Bay. Furthermore, we assumed that 10% of the water gets into Waconda Bay for the model study. We also estimated that approximately 4.4×10^4 liters s^{-1} (1,000 MGD) of water from the Tennessee River intrudes into

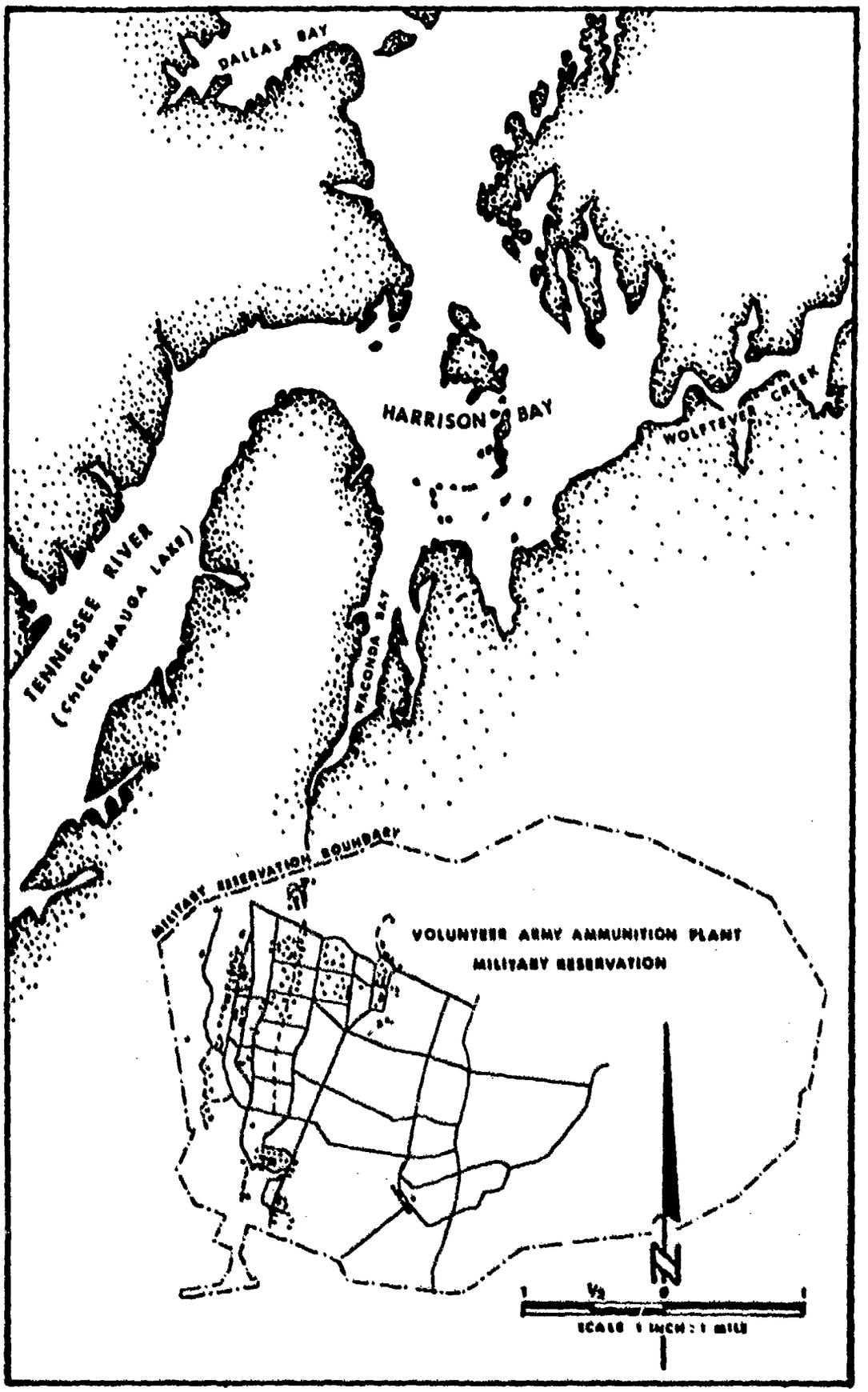


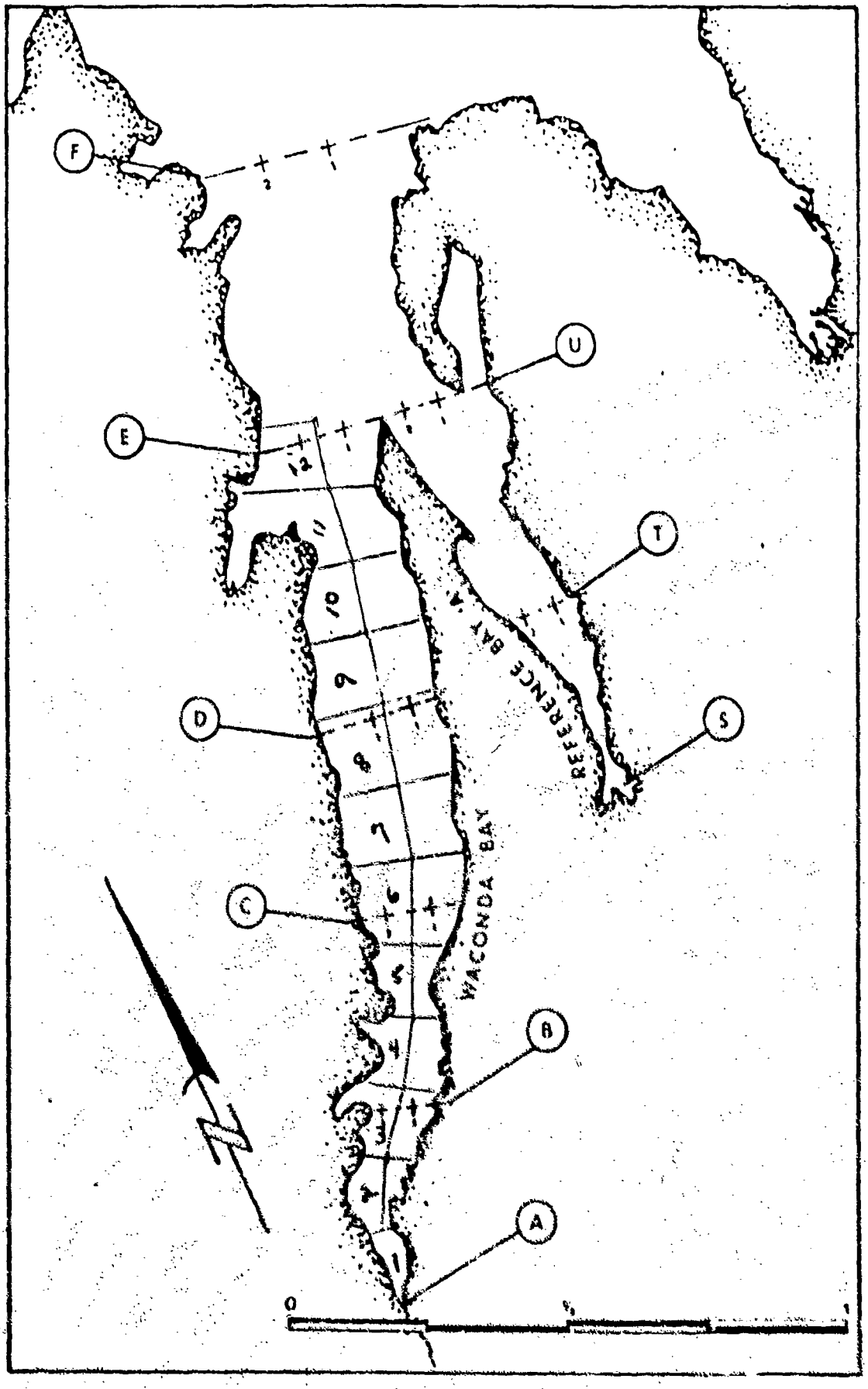
FIGURE B-8 WACONDA BAY AND SURROUNDING WATER BODIES

Waconda Bay, and that the intruding water moves along the south shore of the bay. The amount of returning flow as the intruding water moved into the upper bay was assumed to decrease. This in turn, indicated that the dilution factor also decreased as water moved up into the head of the bay.

Water quality. A water quality survey in Waconda Bay was conducted during two 5-day periods--9-13 June and 11-15 August 1975 (Sullivan, 1977a). Monitoring data in that survey suggested that hardness, sulfate, chlorine, and nitrate levels in the upper bay differed significantly from those of the rest of the bay because of the waste discharge from the Volunteer plant. Suspended solids were reported to be 10 mg liter $^{-1}$ and DO concentrations ranged between 6 and 11 mg liter^{-1}. No microbial population has been reported, but it is believed that microbial activity should be at a normal level.

Compartments selected for the simulation model. Because chemicals had been detected moving along the north side of Waconda Bay and the river water from the Tennessee River intruded into the bay along the south side, Waconda Bay was divided into 12 compartments, as shown in Figure B-9 for the north side of the bay. The hydrologic properties of the north side of Waconda Bay are presented in Tables B-12 and B-13 for discharge rates of 220 liters s^{-1} (5 MGD) and 1100 liters s^{-1} (25 MGD), respectively, if the intruding water rate of 4.4×10^6 liters s^{-1} (1,000 MGD) is assumed to be constant.

Chemical discharges at the Volunteer AAP and Waconda Bay. Production of TNT at the Volunteer plant has been reduced since 1973. It has been reported that the total industrial waste of 1100 liters s^{-1} contains 0.3 to 1.0 mg liter^{-1} of TNT prior to 1973 (Rosenblatt, 1973). In the 1977 study (Sullivan et al., 1977a), the rate of waste discharge was reported to be down to 220 liters s^{-1}. To understand the effect of TNT in Waconda Bay, two TNT loadings were speculated, to depict high- and low-productivity periods. In both cases, 1 mg liter^{-1} of TNT in the waste stream was assumed. This corresponds to 200 mg s^{-1} and



Scale, 25.4 mm = 0.4 km (1 in = 0.25 mile)

FIGURE 8-9 COMPARTMENTALIZATION OF WACONDA BAY

Table B-12

INTERFLOWS AND RESIDENCE TIMES IF WASTE DISCHARGED FROM THE VAAP AT 1100 liter s^{-1}

Compartment Number	Flow Rate		Residence Time (Days)
	liters s^{-1}	10^{-5} × liters day^{-1} × 10^{-10}	
1	0.055	0.047	0.020
2	0.077	0.066	0.014
3	0.098	0.084	0.024
4	0.120	0.104	0.033
5	0.142	0.123	0.047
6	0.186	0.160	0.045
7	0.229	0.198	0.050
8	0.273	0.236	0.042
9	0.317	0.274	0.035
10	0.361	0.312	0.030
11	0.405	0.350	0.034
12	0.449	0.388	0.030

Table B-13

HYDROLOGIC PROPERTIES OF THE NORTH SIDE OF WACONDA BAY IF WASTE DISCHARGED FROM THE VAAP AT 220 LITER s^{-1}, ASSUMING 4.4×10^4 liter s^{-1} OF INTRUDING WATER FROM THE TENNESSEE RIVER

Compartment Number	Surface Area $m^2 \times 10^{-3}$	Depth m	Volume $m^3 \times 10^{-2}$	Flow Rate		Residence Time (Days)
				Liters $Day^{-1} \times 10^{-9}$	Liters $s^{-1} \times 10^{-5}$	
1	16	0.6	9.6	$.40 \times 10^9$	0.046	0.024
2	10.6	0.9	9.5	$.59 \times 10^9$	0.068	0.016
3	23	0.9	20.7	$.78 \times 10^9$	0.090	0.026
4	23	1.5	34.5	$.96 \times 10^9$	0.11	0.035
5	24	2.4	57	1.2	0.14	0.048
6	24	3	72	1.5	0.17	0.048
7	33	3	99	1.9	0.22	0.052
8	33	3	99	2.3	0.27	0.043
9	32	3	96	2.6	0.30	0.035
10	32	3	96	3.0	0.35	0.032
11	40	3	120	3.4	0.40	0.035
12	40	3	120	3.8	0.44	0.031

1,000 mg s^{-1} of TNT being discharged from the plant for 220 liters s^{-1} and 1100 liters s^{-1} discharge rates, respectively. The waste water passes through holding ponds and drains northward through a narrow and shallow creek to the plant boundary. The traveling times of waste water from the plant to the discharge point at Waconda Bay were assumed to be 1 hour and 0.2 hour for the 220 liters s^{-1} and 1100 liters s^{-1} discharge rates, so the TNT concentration at the discharge point should be 0.25 mg $liter^{-1}$ and 0.75 mg $liter^{-1}$. In this study, the TNT loadings at the discharge point located at the head of the bay were estimated to be about 55 mg s^{-1} for low and high discharge rates.

Estimate of 2,4-DNT loading to Waconda Bay. Concentrations of 2,4-DNT in the wastewater stream from the Volunteer Plant were reported to be in the range of 1 to 40 ppm (Spanggord et al., 1978). The high concentration of 2,4-DNT results from the condensate wastewater from the TNT purification process. Therefore, the ratio of 2,4-DNT to TNT in the wastewater stream of the Volunteer plant was expected to be higher than that in the waste stream from the Radford plant. Because the photolysis and biodegradation half-lives of 2,4-DNT were found to be at least several hours, much longer than the traveling time of waste water from the plant to the head of the bay, only a small percent of the 2,4-DNT was believed to be transformed before it reached the bay. Therefore, 2,4-DNT loadings at the head of Waconda Bay were estimated to be 8.8×10^3 mg s^{-1} and 43.8×10^3 mg s^{-1} for the waste water discharge rates of 220 and 1100 liters s^{-1}, respectively.

Monitoring data in Waconda Bay. In the WAR, Inc. survey (Sullivan, 1977a) of two 5-day periods in 1977, 2,4-DNT monitoring data are tabulated in Tables B-14 and B-15; 2,4-DNT was found in the west side of the bay even beyond compartment 12. Concentrations of 2,4-DNT were reported to be over 100 ppb at the head of the bay to over 10 ppb in the lower bay (see Figure B-9 for sampling locations).

Table B-14

CONCENTRATIONS OF 2,4-DNT (ppb) FOUND IN WACONDA BAY DURING 9-13 JUNE 1975*

Sampling Location (See Fig. B-9)	Date of Monitoring, 1975				
	9 June	10 June	11 June	12 June	13 June
A	40	0	130	172	0
B-1	19	56	26	52	87
B-2	0	0	80	137	114
C-1	0	< 2	< 2	< 2	< 2
C-2	< 2	0	31	51	52
D-1	0	< 2	< 2	0	< 2
D-2	16	40	19	32	17
E-1	< 2	0	0	0	< 2
E-2	0	0	0	0	0
F-1	0	0	0	0	---
F-2	12	0	10	0	13

*Source: Sullivan et al., 1977.

Table B-15

CONCENTRATIONS OF 2,4-DNT (ppb) FOUND IN WACONDA BAY DURING 11-15 AUGUST 1975[a]

Sampling Location	Date of Monitoring, 1975				
	11 Aug	12 Aug	13 Aug	14 Aug	15 Aug
A	12	71	17	58	--
B-1	30	< 2	< 2	< 2	45
B-2	51	86	45	27	62
C-1	8	0	44	38	42
C-2	27	< 2	11	< 2	28
D-1	0	0	0	0	--
D-2	15	< 2	< 2	0	0
E-1	< 2	0	< 2	0	0
E-2	0	< 2	0	0	0
F-1	0	0	0	0	0
F-2	15	20	--	0	< 2

[a]Source: Sullivan et al., 1977.

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